CHARACTERIZATION AND GENETIC ANALYSIS OF A VERY HIGH TILLERING AND DWARF RICE (*ORYZA SATIVA* L.) MUTANT

A Thesis

by

DHANANJAY MANI

Submitted to Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2008

Major Subject: Plant Breeding

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Approved by:

Co-Chairs of Committee,	Rodante E. Tabien
	Scott A. Finlayson
Committee Member,	William D. Park
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ABSTRACT

Characterization and Genetic Analysis of a Very High Tillering and Dwarf Rice (*Oryza sativa* L.) Mutant. (August 2008)

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Co-Chairs of Advisory Committee: Dr. Rodante E. Tabien
Dr. Scott A. Finlayson

This study focused on characterizing and determining the inheritance pattern of very high tillering and dwarf traits of a rice mutant. To characterize the new mutant, field phenotyping studies, and response of two mutant lines (M-13662 & M-13684) to three levels of nitrogen (179, 202, 224 kg ha^{-1}) and five planting densities (1, 2, 3, 4, 5 plants $hill^{-1}$) in greenhouse conditions were conducted. A separate study was carried out to determine the response of the two mutant lines to gibberellic acid (GA) application. The mutants were 50-55 cm tall and produced 89-121 tillers $plant^{-1}$ at harvest. Dwarfness of the mutants was due to average shortening of the top four internodes as well as compression of 2-3 basal internodes. The first tiller emerged at the 4^{th} leaf stage whereas no tiller was observed in semi-dwarf rice cultivar, Cocodrie. Results showed that the production of high tiller numbers was the result of the release of axillary buds from a dormant stage rather than the initiation of additional axillary buds. The mutants were late maturing than controls (Cocodrie & Zhe733). The panicles were very short (10-12 cm) and had 25-30 small grains. The majority of tillers of the mutants followed the dn-type dwarf pattern based on Takeda's classification, but a few plants had a

different dwarfing pattern not included in the classification. Both mutant lines were found to have similar agronomic traits but were significantly different from controls. The tillering ability of the mutants was affected by the five different planting densities as well as the three nitrogen levels. Mutants produced more tillers, both productive and non-productive, at the lowest plant density. The longest and shortest panicles were observed at 202 kg ha^{-1} and 179 kg ha^{-1}, respectively. Variations in other agronomic traits were found not significant. The response of the mutant to GA application was similar to Cocodrie, and thus was considered GA responsive. Preliminary DNA data using SSR markers supported the presumed origin of the mutants and the genetic analysis indicated that one recessive gene controlled both the dwarfing and very high tillering traits.

This thesis is dedicated

to my grandfather, grandmother, mom, and dad.

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CHAPTER I

INTRODUCTION

1.1 DISTRIBUTION AND IMPORTANCE OF RICE: Rice (*Oryza sativa* L.*)* is one of the most important staple food crops of Asia, Africa, and South America, and serves as a primary source of food for more than half of the world population (Khush, 2005). It is the main source of the 35-60% dietary calories consumed by more than 3 billion people (Fageria et al., 2003). It is considered as the world's most diverse crop and is probably the most versatile crop. It is grown below sea level in Kerala, India, at more than 3000 m elevation in the Himalayas, and at sea level in the deltas of the Asian rivers. It can be found from 53^0 North in Northeastern China to 35^0 South in New South Wales, Australia. (Mae, 1997; Santos et al., 2003). There are two species of domestic rice, *Oryza sativa* and *Oryza glaberrima. Oryza sativa* is cultivated throughout the world but *Oryza glaberrima* is cultivated mostly in West Africa. *Oryza sativa* is further classified into three sub-species based on geographical distribution and morphological traits: japonica, indica, and javanica (Takahashi, 1984). Japonica and indica are mainly grown in temperate and tropical/sub-tropical areas, respectively. Javanica is also known as "tropical japonica" (Mae, 1997) commonly grown in the U.S. The first trial planting of rice in the U.S. was established in Virginia in 1609 but the commercial cultivation started in South Carolina in the 17^{th} century. Today, rice is being grown in six states: Arkansas, California, Louisiana, Mississippi, Missouri, and Texas.

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This thesis follows the style and format of Crop Science.

Based on grain type, U.S. rice can be classified as long grain, medium grain and short grain. Long grain rice is usually grown in the Southern states with a small amount in California while medium grain rice is generally grown in Arkansas, California, and Louisiana. Short grain rice is mainly produced in California. In 2007, the USDA-ERS reported that 72% of total U.S. rice production was long grain rice. USA is the fourth largest rice exporting country. Total rice production including long, medium, and short grain was 197,911 (1000 cwt) in 2007 (Childs, 2007) and Texas contributed 4.94% of total U.S. rice production. Similar to Asian countries, total U.S. rice harvested area is decreasing. The total U.S. rice planted area was 2748 (1000 acres) in 2007, which was 3.17%, less than total planted area in 2006. Only 5.31% of the planted areas were from Texas in 2007. The average grain yield per hectare of Texas (7,499.90 kg ha^{-1}) ranks fifth after California (9,346.90 kg ha^{-1}), Mississippi (8,227.50 kg ha^{-1}), Arkansas (8,059.60 kg ha^{-1}), and Missouri (7,611.83 kg ha^{-1}) according to the 2007 survey taken by the Economic Research Service, USDA. Due to the exponential rate of population growth, it is estimated that a 40% increase in rice yield is needed by 2030 to fulfill the growing demand without affecting the resource base (Khush, 2005). The agricultural land for crop production is decreasing annually due to urban growth and land degradation, hence, rice production needs to be increased from the same or even smaller amount of land. Novel high yielding rice cultivars, ultra-modern rice production practices and technologies need to be developed to meet the increasing rice demand to feed the entire world. Today's situation is similar to the conditions that started 'Green Revolution' in the late 1960's that fed the increasing human population by planting

semi-dwarf, nitrogen responsive, and disease resistant cultivars of wheat and rice (Peng et al., 1999).

Tillering, plant height and panicle morphology are very important agronomic traits that determine grain production of rice. The total number of tillers includes both productive and non-productive tillers. The number of productive tillers determines the number of panicles that eventually affects the yield and total production of the crop. Plant height is mainly determined by the pattern of internode and panicle elongation and it is dependent on cultivars and the environment. Rice, like most of the gramineous plants, shows internode elongation at a particular developmental stage. Upper internodes start successive elongation, while the rest of the lower internodes remain as unelongated during panicle formation in early maturing rice cultivars but in the late maturing cultivars, the internode elongation precedes panicle formation. Therefore, exploring the relationship between internode elongation and the number of internodes is necessary in each of the cultivars (Takeda, 1977). Dwarfing genes play a very important role in reducing the internode length and/or the number of elongated internodes that affect crop production. The introduction of the semi-dwarfing gene in wheat and rice started the Green Revolution (Peng et al., 1999).

Different kinds of rice mutants have been identified and included in rice breeding programs as germplasm sources and new ones were introduced as resources for new gene discovery, such as the *tos*17, a T-DNA mutant panel (Hirochika et al., 2004). Generally, a reduction of culm length leads to shortening of panicle length in cereal crops but the panicle length is highly correlated to the upper internodes than to lower

ones in rice. The reduction of the elongation of the third and fourth internodes from the top had very little effect on the reduction of panicle length (Takeda, 1975; Takeda, 1977). Many dwarf rice mutants had pleiotropic phenotypes, such as small panicles, small grains, profuse tillers and deformed leaf shapes. These high tillering dwarf rice mutants, although identified a long time ago, were usually not fully characterized because of their poor appearance, several weaknesses, and lack of their economic importance. Earlier dwarf mutants and normal rice cultivars were classified into six groups (N, dn-, dm-, d6-, nl- and sh type) based on the elongation pattern of the upper four internodes (Takahashi and Takeda, 1969; Takeda, 1977). Since the 1980s, a number of different kinds of rice mutants were identified and characterized to establish the relationship between the genes responsible for that particular morphology and the phenotype (Itoh et al., 2005). Therefore, identification and characterization of the different kinds of mutants will be useful in determining the function of genes of the completely sequenced rice genome.

A high tillering dwarf rice mutant was recently identified in a segregating population grown in Beaumont, Texas. This mutant is dwarf and has tremendous capacity to produce very high numbers of productive tillers. High numbers of productive tillers and plant height are two important agronomic traits in several crops and the relationship between high tillering and height is not fully elucidated (Zou et al., 2005). Therefore, this mutant can be a useful genotype in understanding the genetics of these traits and their relationship to one another. Hence, this study aimed to phenotypically characterize the mutant, determine the mutant's response to different levels of nitrogen,

population densities, growth regulator, and study the pattern of inheritance of the observed phenotypes.

1.2 MORPHOLOGY OF THE RICE PLANT: The rice plant, *Oryza sativa*, belongs to the grass family. Rice plant growth is mainly divided into three different stages: vegetative, reproductive, and grain filling or ripening stages (Counce et al., 2000). Germination, emergence, leaf production, seedling establishment and tiller production occur in the vegetative stage of the plant life cycle. The reproductive stage includes culm elongation, the emergence of the flag leaves, booting, heading, and flowering. The ripening stage of rice is defined as grain filling or hardening of the grains. Grains contain the lowest amount of moisture at the ripening stage. The whole rice plant is divided into three vegetative parts: root, culm and leaf.

1.2.1 Root: The root of the rice seedling includes the radicle (seminal root), the mesocotyl root, and nodal root. The coleorhiza, a covering of the radicle, protrudes from the seed first during aerobic seed-germination. If the seeds germinate in anaerobic conditions (in water), the coleoptile, a covering of young shoot, protrudes first followed by coleorhiza emergence. The rice root system is basically composed of adventitious roots (Yoshida, 1981). The root or soil environment has an important role in the formation of root hairs which are mainly responsible for the absorption of water and nutrients. The root system of upland or aerobic rice is larger, more vigorous, and has more root hairs as compared to anaerobic flooded or lowland rice. The root growth to maximum root length was estimated using a quadratic function with the advancement of plant age from 19 to 120 days after sowing (Fageria, 2007).

1.2.2 Culm: The mesocotyl is a structure which helps the coleoptile reach above the soil surface. The culm is cylindrical and hollow except at the nodes. The node or nodal region bears a leaf and a single bud.

1.2.3 Leaf: Leaf morphology such as length, width, erectness, thickness, and toughness are very important characteristics in determining the yield capacity of a cultivar. Erect leaves allow more uniform distribution of light in the plant canopy and increases photosynthetic efficiency of the plant. The normal rice leaf consists of a leaf sheath, auricles, and a leaf blade. The first leaf has no leaf blade but the second leaf is a true leaf with leaf blade and leaf sheath. The remaining leaves of the rice plant are normal, except the flag leaf, which is the topmost, or the last leaf produced on the main stem. The flag leaf supplies the photosynthetic products to the developing panicles. The collar is a structure which joins the leaf blade and leaf sheath. The leaf has parallel veination. Auricles are ear-like structures at the base of the blade while the ligule is a leaf appendage which is present at the junction of the leaf sheath and the leaf blade. Active buds in the leaf axils produce tillers. Primary tillers are produced from the mother tiller (main culm) and may produce secondary tillers, which then produce tertiary tillers later. Primary tillers are produced in an alternate manner on the main culm.

CHAPTER II

REVIEW OF LITERATURE

2.1 TILLER FORMATION: Plant architecture is mainly characterized by tiller number, tiller angle, plant height, and panicle morphology (Wang and Li, 2005). The shoot apical meristem (SAM) has a very important role in the production of axillary branches. It is the source of leaves and tillers (Li, 1979; Wang and Li, 2005). Tillers arise from the axillary meristems which are present in the leaf axils of the plant. Tiller formation depends upon the initiation of axillary meristems in the leaf axil of a leaf, and also upon its subsequent activity (Wang and Li, 2005). The shoot apex varies greatly in size and shapes. The shape, which depends upon the species and the genetic make-up of different plants, may be elongated, conical, dome shaped, flat or even slightly concave. The SAM consists of a number of the pluripotent stem cells, which have different functions. There are three different zones in the SAM: the central zone, the peripheral zone, and the rib zone. The central zone is considered as the reservoir of pluripotent stem cells where slow cell division has been observed. The peripheral zone surrounds the central zone where cell division is relatively faster than the central zone. In the third zone, the rib zone, the rate of cell division is similar to the peripheral zone. The leaves originated from the peripheral zone and the rib zone are responsible for the stem formation (Bowman and Eshed, 2000).

2.2 TILLERING IN RICE: The branches of rice are known as tillers. Tiller buds of rice are axillary buds, which are formed in the leaf axils and produce tillers after differentiation of the axillary buds. The mother culm is the source of nutrients for tiller

buds inside the leaf sheath, but after the third leaf stage tillers start their own photosynthesis and their mode of nutrition shifts to autotrophy from heterotrophy (Hanada, 1995). Usually, tillering begins 15 to 20 days after germination (4^{th} or 5^{th} leaf stage) under favorable conditions and emergence of tillers is closely associated with the number of leaves. During the tillering stage, the rate of protein synthesis was higher as compared to synthesis of starch, lignin, and cellulose (Hayashi, 1995). Rice tillering ability was affected by environmental conditions such as light, temperature, plant density, and nutrients (Wu et al., 1998). Although it was affected by environmental factors, the tiller number of a particular rice cultivar was mainly determined by its genetic make-up (Wang and Li, 2005).

2.3 RELATIONSHIP BETWEEN TILLERS AND YIELD: Tillering is a very important agronomic trait under biotic and abiotic stresses due to compensation processes. More than 75% of the total mass was represented by tillers in the high tillering cultivar (Teqing) at lower plant density, compared with 71% and 69% for the moderate tillering cultivars, Gulfmont and Rosemont, respectively (Wu et al., 1998). Fageria (2007) also reported that high tillering cultivars were better than low tillering cultivars, especially at lower plant densities and unfavorable environmental conditions, because high tillering cultivars compensate the yield for missing plants at low densities by producing more tillers. However, under favorable environmental conditions, there was no significant advantage among very high tillering cultivars and low tillering cultivars in relation to yield. The tillering ability of the rice plant had a great impact on panicle production (Miller et al., 1991), which was highly correlated with grain yield

(Counce and Wells, 1990; Miller et al., 1991). Rice grain yield was increased as final tiller density increased up to 700 tillers m^{-2} in a continuously flooded and water-seeded cultural system (Miller et al., 1991). A recent study has also shown that the number of tillers determined at the initiation of panicle growth stage was more highly correlated with grain yield than at any other growth stages in lowland rice (Fageria, 2007). In spring wheat grown in Saskatoon, Hucl and Baker (1989) found that 67% of the grain yield of spring wheat was from the main stem and primary tillers. Recently, Goos and Johnson (2001) reported that the main stem and primary tillers contributed 95 to 100% of the grain yield of hard red spring wheat.

2.4 FACTORS AFFECTING TILLERING AND GRAIN YIELD IN RICE

2.4.1 Nitrogen: Nitrogen is one of the most important nutrients for the rice plant because it is associated with chloroplast and protein synthesis, which are physiologically important in dry matter production (Dalling, 1985). However, nitrogen represents one of the most expensive production inputs, and it is the most limiting nutrient in flooded as well as upland non-flooded rice production worldwide (Becker et al., 1994; Baligar and Fageria, 1997). Nitrogen plays an important role in carbohydrate accumulation in culms and leaf sheaths during the booting stage and in the grains during the grain-filling stage. The amount of fertilizers needed during the growth of the plant depends on the type of fertilizers, soil type, rice cultivars, climate, and methods of application (Mae, 1997). Nitrogen rates for optimum grain yield vary according to cultivar and soil texture (Norman et al., 2005; Bond et al., 2006). Additional doses of nitrogen were needed during grain filling because nitrogen applied at the early stage or from mineralization of

labile soil organic matter has already been used to promote early growth and to increase the number of tillers by the maximum tillering stage (Mae, 1997). Higher doses of nitrogen at the early stage promote excessive vegetative growth, create lodging problems and increase the incidence of foliar pathogens (Bohlool et al., 1992), but topdressing of nitrogen at the late phase of panicle formation increases the crop yield rather than promoting lodging (Mae, 1997). Topdressing of nitrogen at the heading stage is very important to improve the grain yield since rice plants actively absorb nitrogen until two weeks after heading and higher nitrogen doses along with elevated carbon dioxide concentrations led to higher tiller number production and higher biomass plant^{-1} (Weerakoon et al., 1999). Early tiller production was influenced by the nitrogen level and timing of nitrogen application in no-till water-seeded rice but independent from the nitrogen timing and the amount of nitrogen interaction (Stevens et al., 2001). The application of nitrogen fertilizer in either excess amounts or less than optimum rates affected both yield and quality of rice (Liu, 1991; Saito, 1991).

Mossedaq and Smith (1994) reported that the growth and development of spring wheat was dependent on the rate and timing of the nitrogen fertilizer application. It was found that the nitrogen demand was abruptly increased just before stem elongation during crop growth. In rice, Fageria and Baligar (1999) reported that the rate and timing of nitrogen fertilizer application significantly affected the grain yield as well as dry matter accumulation of lowland rice. Fageria and Baligar (1996) observed in central Brazil on Varzea soil that lowland rice yields were significantly higher at 200 kg N ha^{-1} than at 100 kg N ha^{-1}. The effect of nitrogen application varied with fertilization time

(Bacon and Lewin, 1990). Usually, the nitrogen is applied to rice crop in three split applications. It allows for more efficient nitrogen use at different stages of plant growth as it provides specific amounts of nutrients throughout the growing season and reduces leaching of nitrate in the soil. Strong (1986) reported that tiller production was increased when nitrogen was applied before planting or during the tillering process in spring wheat. Therefore, optimum amounts of nitrogen, timing of application, and method of application must be determined for every crop to achieve potential grain yield. The production of non-productive tillers varies according to the amount of nitrogen applied and cultivars (Amin et al., 2006). The judicious use of available advanced technologies and the development of novel cultivars could help achieve desired yield potentials for any crop.

2.4.2 Density: Seeding rate is also one of the principal factors affecting tiller production capacity (Counce et al.,1992) and the numbers of total tillers and stems increased with increasing planting density, while the numbers of secondary and tertiary tillers decreased with increasing planting density in all rice cultivars evaluated (Nuruzzaman et al., 2000). Plant density was found inversely proportional to the number of secondary and tertiary tillers (Hoshikawa, 1989; Wu et al., 1998). The number of productive tiller is a very important agronomic trait, however, it is affected by planting density. Ottis and Talbert (2005) reported that the number of productive tillers was decreased at higher planting density. Nitrogen and plant density play important roles in the production of tiller and eventually, yield of the crop. Higher seeding rate increased tiller density but produced low number of grains spike^{-1} in wheat (Done and

Whittington, 1980). In rice, tiller density increased significantly with increasing plant density from 122 to 458 plants m^{-2}, while total biomass above the ground was not significantly different among the plant populations (Miller et al., 1991). Tiller abortion rates was increased by higher tiller number and a highly significant negative correlation (r = -0.86**) was found between percentage of productive tillers and maximum tiller number (Schnier et al., 1990).

2.4.3 Planting date: Planting date has an important role in tiller production apart from nitrogen and planting density. Tiller formation was reduced in hard red spring wheat which, resulted in a significant reduction of spikes m^{-2} in the case of delayed planting (Black and Siddoway, 1977) but on the other hand, more tillers were observed in early planting which resulted in a significant increase of spikes m^{-2} (Hucl and Baker, 1989). In rice, delayed planting significantly reduced the grain yield by 0.88 t ha^{-1} in aromatic rice (Ghosh et al., 2004)), however, high planting density can compensate for the yield loss caused by the late planting (Baloch et al., 2006).

2.4.4 Growth regulators: The activity of axillary buds is controlled by multiple genetic and developmental or environmental signals, and apical dominance that suppresses the growth of axillary bud is one of those signals (Zou et al., 2006). It was first demonstrated that the removal of the shoot apex, a major site for auxin biosynthesis, facilitated the growth of dormant axillary buds (Thimann and Skoog, 1933). Auxin promotes apical shoot dominance, which inhibits axillary bud activity, and on the other hand, cytokinin promotes the axillary bud outgrowth. The role of cytokinin in shoot

production was supported by the high levels of cytokinin and bushy phenotype in the different *Arabidopsis* mutant studies (Chaudhary et al., 1993; Catterou et al., 2002).

2.5. FACTORS AFFECTING PLANT HEIGHT, PANICLE DENSITY AND GRAIN YIELD: Rice plant height is an important agronomic trait because it improves harvest index and it is associated with plant lodging. Semi-dwarf plant stature increases harvest index (grain/grain plus straw) and enhances biomass production (Khush, 1999). Plant height is defined as the distance from ground level to the tip of the tallest leaf for seedling but it is the distance from ground level to the tip of the tallest panicle at harvest (Fageria, 2007). It is determined by the total number and length of internodes and varies according to genetic make-up of the plant and environmental condition (Wang and Li, 2005). Qualitative genes and quantitative loci were associated with plant height (Huang et al., 1996). The success of green revolution was associated with the semi-dwarf cultivars of wheat and rice that were very responsive to heavy doses of fertilizer (Yoshida, 1981; Khush, 1999; Peng et al., 1999).

2.5.1 Nitrogen: Nitrogen fertilizer is essential for higher grain production, but it also promotes leaf and stem elongation, which enhance plant stature. Plant heights, productive tillers $hill^{-1}$ and panicle length were positively correlated with higher doses of nitrogen (Manzoor et al., 2006). Plant height, number of productive tillers $hill^{-1}$, panicle length, number of grains $panicle^{-1}$, 1000-grain weight, and yield increased from 0 kg ha^{-1} to 175 kg ha^{-1} in the Basmati 2000 rice cultivar. On the other hand, total yield, number of grains $panicle^{-1}$ and 1000-grain weight started declining beyond the 175 kg N ha^{-1}. Higher panicle density was the result of higher dose of nitrogen application (Bond et al.,

2008). Similarly in wheat (*Triticum aestivum L.),* nitrogen increased plant height and the number of grains spike^{-1} (Khan et al., 2000; Iqtidar et al., 2006).

Spikelet sterility is not desirable in crop improvement program since it reduces the grain yield and it depends upon the cultivar as well as nitrogen level (Fageria, 2007). Rice grain yield can be increased by as much as 15% if rice breeding eliminates spikelet sterility. The application of adequate amount of nitrogen accounted for about 91% variation in panicles m^{-2}, approximately 75% variation in spikelet sterility, and about 73% variation in 1000 grain weight in lowland rice (Fageria and Baligar, 2001; Fageria, 2007). The amount of nitrogen and timing of application had a major role in improving grain yields. High yielding rice cultivars needed relatively higher amounts of nitrogen than average yielding rice cultivars (Wada et al., 1986). However, the formation of each yield component was not only dependent on the absolute amount of nitrogen but it was also dependent upon the nitrogen supply pattern and uptake process at each growth stage for respective yield component (Mae, 1997). Nitrogen applied during booting or flowering stage did not improve grain yield but it kept rice leaves more green during the grain filling growth stage. The number of panicles and grains were already fixed when the nitrogen was applied at the reproductive stage (Castillo et al., 1992; Fageria and Baligar, 1999). Thousand grain weight did not change significantly with nitrogen treatments and was a very stable varietal character under different growing conditions (Yoshida, 1981; Fageria and Baligar, 1999).

2.5.2 Density: Agronomic factors such as plant height and grain yield are highly affected by plant spacing in rice crop. Plant spacing varies according to cultivars. Short

stature or semi-dwarf cultivars had higher yields at close plant spacing compared to taller cultivars (Tanaka et al., 1964). Dofing and Knight (1994) reported that taller plant height and weaker culms were due to higher plant densities and this may increase the potential losses due to lodging and disease in barley. Wells and Faw (1978) reported that seeding rates had no significant effect on yield at low nitrogen levels, but lower seeding rates had significantly higher yields at high nitrogen dose. An inverse relationship was found between panicle size and panicle density because the source becomes a limiting factor to fill large sink size, primarily in large number of panicles per unit area (Fageria, 2007). There was also an inverse relationship between the percentage of ripened spikelet and the panicle density (Yoshida, 1981). It was reported that as rice seedling rates increased, filled grains panicle^{-1} decreased with no changes in yield (Jones and Synder, 1987; Gravois and Helms, 1992). Fageria and Baligar (1999) reported that spikelet sterility was increased as the number of panicles or number of spikelets per unit area increased and it was attributed to imbalance between higher sink (spikelet) capacities and comparatively lower source capacity (photosynthesis). Panicle density, spikelet panicle^{-1}, weight of spikelet, and grain filling were the main yield components and panicle density was responsible for the highest variation in grain yield (Fageria, 2007). A negative correlation was found between spikelet sterility and grain yield (Fageria and Baligar, 1999) and a high positive correlation was found between panicle density and total grain yield (Ottis and Talbert, 2005). Fageria (2007) reported that panicle density had a major role in determining the total grain yield. Final tiller density was an important factor in determining rice grain yield in a flooded, water-seeded cultural system with the

maximum yield reported at 700 tillers m^{-2} (Miller et al., 1991). Therefore, there must be an appropriate number of panicles and plants per unit area to achieve the maximum yield.

2.5.3. Growth regulators: Two growth regulators, gibberellic acid (GA) and brassinosteroid (BR) are known to play major roles in controlling rice plant height (Yamamuro et al., 2000; Sasaki et al., 2002; Wang and Li, 2005). GA was first isolated by Cross from the *Fusarium moniliforme* Sheldon stage of *Gibberella fujikuroi* (Saw.) Wr (Phinney, 1956). GA plays a very important role in promoting cell elongation in a number of higher plants and induces hydrolytic enzymes (α-amylase) in the aleurone layer of cereal seeds. It was found to enhance the cell division process in the inter-calary meristem of submerged deepwater rice by activating histone kinase and cyclin genes during the induction of rapid growth in the internodes (Sauter et al., 1995). GAs are known as a major plant hormone involved in promoting the growth of the rice leaf sheath (Matsukura et al., 1998) and have been found effective in delaying flowering and panicle exsertion important in hybrid rice seed production (Virmani and Sharma, 1993).

Brassinosteroids have a great influence on both plant height and leaf erectness in rice which are very important agronomic traits in crop production. Recently, a rice dwarf mutant, *d61*, was characterized. It was found that the pleiotropic abnormal phenotype of dwarfism and erect leaves was associated with defect in the synthesis of brassinosteroids (Yamamuro et al., 2000).

2.6. DESCRIPTION AND CLASSIFICATION OF DIFFERENT KINDS OF RICE DWARF MUTANTS: Dwarfness plays an important role in lodging resistance. Most of

the semi-dwarf cultivars are high yielding because of their lodging resistance and high harvest index under intensive cultural practices. Several dwarf mutants were identified and characterized in rice but most of them originated from induced mutations (chemicals, radiation). On the other hand, breeding populations or big rice production areas are good sources of natural mutants, but these mutants are usually not identified and kept by the breeders/farmers due to their poor appearance or low agronomic values. There were a number of mutants having abnormal patterns of shoot branching, mostly with defects in axillary meristem initiation or subsequent out-growth or both, that were identified and described in different species such as maize, tomato, and Arabidopsis (Doebley et al., 1995; Schumacher et al., 1999; Stirnberg et al., 1999; Shimizu-Sato and Mori, 2001; Hubbard et al., 2002; Ward and Leyser, 2004). Quantitative trait loci and molecular analysis showed that the *TB1* gene regulates branching in maize (Doebley et al., 1995).

Studies identified many genes in rice, which were involved in the initiation and out-growth of rice tiller buds or leaves during the vegetative stage (Komatsu et al., 2003; Li et al., 2003; Takeda et al., 2003). *LAX* and *SPA* were identified as major regulators of axillary meristem formation in rice and the mutants (*lax & spa*) with these traits had reduced number of panicle branches because of the suppression of initiation of lateral branches (Komatsu et al., 2003).

The dwarf stature of plants is highly associated with GAs. The Green revolution genes, *wheat reduced height1* (*Rht1*) and rice *semi-dwarf1* (*sd-1*) were involved in GA signaling and GA biosynthesis, respectively (Peng et al., 1999; Sasaki et al., 2002;

Spielmeyer et al., 2002). The *sd-1*, a recessive, semi-dwarfing gene, is one of the most important genes used in rice breeding program and was first identified in the Chinese rice cultivar 'Dee-geo-woo-gen'. Presence of this gene resulted in a shortened culm with high lodging resistance and a greater harvest index, allowing for increased use of nitrogen fertilizers (Jennings, 1964). The green revolution rice cultivar IR8 was developed by crossing Dee-geo-woo-gen with the *sd-1 gene* and 'Peta' (tall) in 1960 (IRRI, 1967). This cultivar produced record yields throughout Asia and formed the basis for the development of new high yielding, semi-dwarf plant types. Identification and characterization of the Green revolution gene (*sd1*) opened the door for the development of high-yielding semi-dwarf cultivars. After IR8 release, many semi-dwarf rice cultivars were developed and released due to the agronomic importance of this trait in the breeding programs.

Extensive studies have been carried out to elucidate the dwarfing mechanism in rice dwarf mutants and to identify the genes involved. The first cloned rice dwarf mutant gene was *d1* and its dwarfing phenotype was associated with non-production of a functional GTP-binding protein involved in GA signal transduction (Ashikari et al., 1999). The two rice dwarf genes, *d61* and *d2,* failed to synthesize and perceive brassinosteroid (BR), respectively (Yamamuro et al., 2000; Hong et al., 2003). The *d2* mutant was only 70-80% of the height of the wild-type plant and the dwarfness of this mutant was due to complete or partially shortening of the second internode from the top. The elongation of other internodes was found not affected in this mutant (Hong et al.,

2003). On the other hand, the two types of internode elongation (dm-type and d6-type) were observed in the *d61* mutant (Yamamuro et al., 2000).

Takeda (1977) classified dwarf mutants into six groups, based on the elongation pattern of the top four internodes. The N represents the internode elongation of the normal strains, whose internodes generally become shorter from the top internode to the basal internode. The dn-type represents the elongation pattern of some dwarf strains whose successive internodes are reduced uniformly. On the other hand, dm-type and the sh-type mutants show reduced length of the second internode and first uppermost internode, respectively. The nl-type shows a dual effect; reducing the length of the uppermost internode, and increasing the length of basal internodes. The d6 is another type whose uppermost internode measures more than half of the total culm length. Another classification of rice dwarf mutants given by Mitsunaga et al. (1994) was based on the growth response to gibberellic acid (GA) application. Dwarf rice mutants were classified into three groups: T, D, and E. The T group was represented the GA-deficient mutants and the D group was comprised of GA-insensitive mutants, whereas those mutants that were neither GA-deficient nor GA-insensitive represented the E group.

2.6.1 Mutants related to tillering: Besides the semi-dwarf habit, tillering is another important agronomic trait that affects panicle production. Therefore, several rice tillering mutants were identified, but most of them are not completely characterized (Goto et al., 2005). Tillering dwarf mutants were characterized by an increase in tiller number as well as a reduction in plant height (Kinoshita and Takahashi, 1991). Five tillering dwarf rice mutants (*d3, d10, d14, d17* and *d27*) that showed reduced plant

height and increased tiller number have been characterized and this phenotype was the result of decreased suppression of tiller bud out-growth (Ishikawa et al., 2005). Recently, rice '*fine culm 1*' (*fc1*) mutant and a single tiller mutant called '*monoculm 1*' mutant (*moc1*) were identified (Li et al., 2003; Takeda et al., 2003). The *fc1* mutant produced twice as many tillers as the wild-type rice plant and the pattern of flowering was unaffected (Goto et al., 2005). Both the *d* mutants (*d3, d10, d14, d17* and *d27*) and the *fc1* mutants were dwarf and high tillering but both mutants differed from each other in dwarfing pattern. Dwarfness of *d* mutants was accounted to suppression of all internodes that led to a dwarf phenotype, whereas significant shortening was observed only in the most apical internode in *fc1* (Arite el al., 2007). The absence of tillering ability in *moc1* mutant was due to the loss of the capacity to initiate tiller buds (Li et al., 2003). The *MOC1* locus was mapped on the long arm of chromosome 6. Based on sequence similarity with maize *Teosinte branched1* (*TB1*), which was involved in lateral branching in maize, another rice tillering gene, *TB1* gene (*OsTB1*) was identified (Takeda et al., 2003). It was reported that overproduction of *OsTB1* significantly reduced the lateral branching in rice while its loss-of-function mutation in the mutant *fine culm 1 (fc1)* promoted the out-growth of the rice tillers. The initiation of axillary buds, however, was not affected in *OsTB1* over-expresser transgenic lines. This indicates that the major role of *OsTB1* was to control the out-growth of tiller buds rather than the initiation of tiller buds (Takeda et al., 2003). In addition to regulating apical dominance, the *TB1* gene that was similar in sequence with the *OsTB1* gene of rice was identified to have a role in the development of inflorescence in maize (Doebley et al., 1997). *TB1* is a

member of the TCP binding domain group of transcriptional regulators. Many members of the TCP family were involved in controlling cell division and growth either alone or combining with some other proteins (Cubas et al., 1999). Recently, the *htd-1* (high tillering dwarf 1) allele was discovered in rice, and it was responsible for high tillering trait. The *htd-1* mutant produces excessive number of tillers and the first tillers started coming out from their leaf sheath at the third leaf stage. The *htd-1* mutant enhanced the number of tillers by releasing axillary bud out-growth from the dormant stage rather than by initiating more axillary buds in the leaf axils. GA assays revealed that the mutant *htd-1* and Nanjing 6 (wild rice variety) had the same α-amylase activity and had almost the same ratio of the lengths of the second leaf sheath with and without prior application of GA as Nanjing 6 (control, wild rice variety). This indicated that the mutant *htd-1* was neither GA deficient nor a GA insensitive dwarf (Zou et al., 2005). Genetic analysis revealed that high tillering and dwarf traits of the *htd-1* mutant were controlled by a single recessive nuclear gene, *htd-1* and it was fine mapped in a 30-kb DNA region on chromosome 4 (Zou et al., 2005).

CHAPTER III

MATERIALS AND METHODS

3.1 SOURCE OF MUTANT: A very high tillering dwarf rice mutant was selected from an early generation population of an L-202 x Saber cross developed at Texas Agrilife Research and Extension Center, Beaumont, Texas as shown in Figure 1.

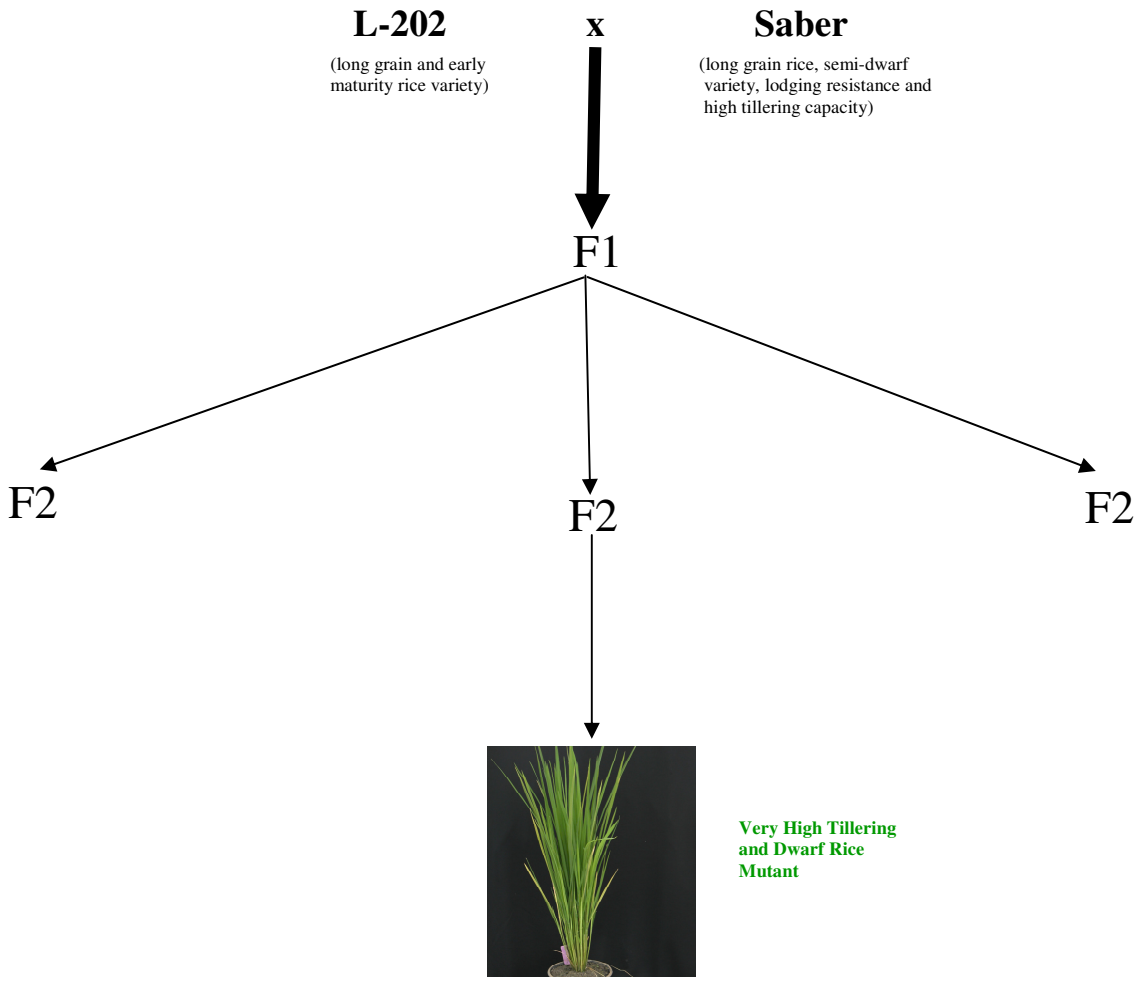


Figure 1. Schematic diagram of origin of the very high tillering and dwarf rice mutant.

'Saber' is a long grain, semi-dwarf rice cultivar having excellent lodging resistance and high tillering capacity (McClung et al., 2004). This cultivar was released by USDA-ARS for production in the Southern growing region of the U.S. L-202 is also a long grain and early maturity rice cultivar. The identified mutant was advanced for four generations, using panicle to row planting under flooded conditions of a China clay soil (fine, smectic, hyperthermic Oxyaquic, Dystrudert). Several sister lines were advanced, but two lines (M-13662 & M-13684) were used for detailed phenotypic characterization.

3.2 PHENOTYPIC CHARACTERIZATION: Two cultivars with the semi-dwarf gene, *sd-1* (Zhe733 & Cocodrie) were chosen as controls to characterize the very high tillering and dwarf rice mutant. Currently, Cocodrie is the most popular cultivar in southern USA. Zhe733 is a fast tillering cultivar and has a high tillering capacity as compared to Cocodrie. Sixteen lines of mutant and two controls were planted in 1.83 m single row plots. Nitrogen fertilizer was applied at the rate of 223 kg ha^{-1} in three splits. The first, second and third doses of nitrogen were applied at the time of planting, flooding, and panicle initiation stage at the rate of 56 kg ha^{-1}, 89 kg ha^{-1}, and 78 kg ha^{-1}, respectively. Phosphorus was also applied at the rate of 17 kg ha^{-1} as a pre-plant treatment. Plants were treated with different kinds of herbicides and insecticides (Command 4EC, Permit, Stam 80 EDF, Bolero 8 EC, Basagram, and Mustang Max) as needed. Five plants were selected from each of the mutant lines for tillering studies along with Zhe733 and Cocodrie as controls. The numbers of tillers $plant^{-1}$ were counted each week starting from 30 days after emergence. The date of 50% heading and maturity were taken. Other agronomic data collected at harvest were as follows:

(a) Number of productive tillers plant^{-1}

(b) Number of non-productive tillers plant^{-1}

(c) Total tiller number plant^{-1}

(d) Percentage (%) of productive tillers

(e) Height (cm)-from the base of the plant to tip of the uppermost fully expanded leaf.

(f) Flag leaf length (cm)

(g) Panicle length (cm)

(h) Filled grains panicle^{-1}

(i) Non-filled grains panicle^{-1}

(j) Ratio of filled grains to non-filled grains

(k) Percentage of grain filling

(l) Total number of grains panicle^{-1}

(m) Grain weight plant^{-1} (g plant^{-1})

(n) Panicle exsertion length (cm)

(o) Seed length (mm)

(p) Seed width (mm)

(q) 1000 grain weight (g)

3.3 DESTRUCTIVE SAMPLING TO DESCRIBE THE TILLERING ABILITY OF THE TWO VERY HIGH TILLERING AND DWARF RICE MUTANT LINES: One hundred and eighty seedlings each for two mutant lines (M-13662 & M-13684) and control cultivars (Cocodrie and Zhe 733) were planted in Jiffy pots for

destructive sampling. After setting up the pots in a big wooden tub, all pots were filled with China clay soil (fine, smectic, hyperthermic Oxyaquic Dystrudert) and the seeds were direct-seeded on each pot. Two weeks after emergence, thinning was done to one plant pot^{-1}. Nitrogen fertilizer was applied as recommended. Destructive sampling was started at the coleoptile stage of rice seedling and was continued until the maximum tillering stage. Three plants of the same stage from each of the mutant and control lines were taken at each sampling date. Destructive sampling was repeated after every 4^{th} day of the previous sampling. At each sampling date, seedlings were uprooted and cleaned with water to get rid of all the soil and then plant height (cm), bud dormancy, 1^{st} tiller emergence, number of primary, secondary, and tertiary tillers were recorded.

3.4 EFFECT OF GIBBERELLIC ACID (GA) ON SECOND LEAF SHEATH ELONGATION: The effect of GA on second leaf sheath elongation was determined by the modified "Microdrop method" of Murakami (1968). Ten seeds of the very high tillering and dwarf rice mutant and control (Cocodrie) were surface-sterilized for 30 min with 10% bleach (NaClO) solution, and then washed three times with sterile distilled water. Seeds were soaked in sterile distilled water for additional 48 h after washing out the bleach. The germinated seeds were placed on top of the solid 1% agar initially poured in a glass tube (Diameter: 2.1 cm, and Depth: 8.2 cm). One seedling was transplanted and maintained in each tube. All planted tubes with 25 ml of 1% agar were arranged in a test tube rack and were kept under fluorescent lamps at 26^0C until GA application. Before GA treatment, GA_3 was dissolved in ethanol, followed by dilution with sterile distilled water to achieve a 200 pmol concentration of GA_3 solution. After

two days, 1µl of GA_3 solution (200 pmol $plant^{-1}$) was applied to the coleoptile of each seedling at the first leaf stage. After 4 days of GA application, the length of the second leaf sheath was measured in each mutant as well as the control cultivar, Cocodrie.

3.5 GENETIC ANALYSIS

3.5.1 Development of F_1 seeds: Flowering plants of the very high tillering and dwarf rice mutants and Cocodrie were balled and potted into black plastic pots early in the morning for emasculation. Five or six panicles were selected for emasculation and the rest of the tillers were removed from the main plant. Emasculation was started at 3:00 pm when the pistil was not receptive. About one third of the floret was cut/detached using sharp scissors during emasculation. Exposed anthers were sucked out using a vacuum emasculator. After removing the anthers, the emasculated panicles were covered using a glassine bag to avoid the introduction of foreign pollen. Panicles of the male parent, the pollinator, were collected at 10:00 am on the next day from the experimental field located at the AgriLife Research and Extension Center, Beaumont, Texas and were placed in a flask with water to avoid desiccation. Once the panicles were releasing pollens, the glassine bags were removed from the emasculated panicles and the panicles of the pollinators were shaken over the top of the emasculated panicles. Care was exercised to avoid the introduction of pollen other than the target male parent. After the introduction of the pollen, the glassine bags were returned, clipped and labeled. The label included the name of the female and male parents, and the date of pollination. Thirty days after pollination, F_1 seeds were harvested, dried and stored in the refrigerator until needed. Crosses including reciprocals were made with Cocodrie to determine the

inheritance pattern of the very high tillering and dwarf trait. Thus, for each mutant, two crosses were made: Cocodrie x mutant, and mutant x Cocodrie. Twenty F_1 seeds were harvested for each cross combination.

3.5.2 Generation of F_2 population: The F_1 plants were grown to generate the F_2 population for genetic analysis. Ten F_1 seeds from each cross combination were treated with Vitavex (fungicide) to avoid fungal infection. Treated seeds were placed in a Petri dish lined with filter paper. Once the seeds germinated and had vigorous roots and shoots, these were transplanted at one seedling pot^{-1} and kept inside the greenhouse. All plants were fertilized as recommended and were sprayed with insecticide when needed. Plants were maintained until maturity. At harvest, panicles from each F_1 plant were gathered, threshed, cleaned and dried at 40^oC in an oven for three days to break seed dormancy. No F_1 survived in one cross, thus only three populations were generated. Seeds were kept inside the refrigerator until needed.

3.5.3 Plants for genetic analysis: Three hundred sixty seven selfed seeds from F_1 plants (F_2 seeds) of M-13662/Cocodrie cross combinations, together with the parents and F_1 seeds were used to determine the segregation ratios of the mutant traits in M-13662, while 413 selfed seeds from the F_1 plants (F_2 seeds) of M-13684/Cocodrie cross combination together with the parents and F_1 were used in M-13684. Four hundred sixty selfed seeds from F_1 plants (F_2 seeds) of Cocodrie/M-13662 with the parents and F_1 were also evaluated as a reciprocal cross for M-13662. The seeds were direct-seeded in black plastic pots (Diameter: 14.8 cm, Depth: 17.5 cm) placed in wooden tubs. Thirty days after emergence, the tubs were flooded. Recommended fertilizers were applied and

insecticides were used if needed. All plants were maintained inside the greenhouse. At maximum tillering (about 45 days after seeding), the number of normal and mutant phenotypes were counted. Harvesting was not done for all plants due to panicle mite infestation and quarantine issues.

3.6 RESPONSE OF THE RICE MUTANT'S AGRONOMIC TRAITS TO VARYING LEVELS OF NITROGEN AND PLANT DENSITY IN GREENHOUSE CONDITIONS: A separate study was conducted in the greenhouse to determine the response of the mutant lines (M-13662 & M-13684) to varying levels of plant densities and nitrogen fertilization. Cocodrie and Zhe733 were used as controls. Three levels of nitrogen (N1=179 kg ha^{-1}, N2=202 kg $ha^{-1}$and N3=224 kg ha^{-1}) and five plant densities (P1=1 plant pot^{-1}, P2=2 plant pot^{-1}, P3=3 plant pot^{-1}, P4=4 plant pot^{-1}, P5=5 plant pot^{-1}) were evaluated to determine their effects on different agronomic traits. Direct seeding was done in black plastic pots (Diameter: 14.8 cm, Depth: 17.5 cm), filled with China clay soil (fine, smectic, hyperthermic Oxyaquic Dystrudert). Each treatment was replicated three times and laid-out in a split plot, completely randomized design. One hundred eighty black plastic pots (Diameter: 14.8 cm, Depth: 17.5 cm) and nine wooden tubs (Fig. 2) were used in this study. Each tub had pots of the same N treatment to avoid contamination with other N treatments when flooded. Nitrogen fertilizer (urea) was applied in three equal splits and that were given at the time of planting, maximum tillering stage and the booting stage. Soil moisture was maintained at field capacity for proper germination of seeds. Thinning was done at the 3rd leaf stage to get the desirable plant density. All the tubs were flooded at the tillering stage (40 days

after planting). Weeding was done from time to time as needed. Sevin, Kelthane, and Orthene were applied to control insects and mites. Plant height (from base of the stem to the tip of the uppermost fully expanded leaf) and total tiller numbers were recorded every week until harvest. Date of flowering and maturity (harvesting) were taken for both mutants and controls. Agronomic data collected at maturity were as follows:

(a) Number of productive tillers plant^{-1}

(b) Number of non-productive tillers plant^{-1}

(c) Total tiller number plant^{-1}

(d) Percentage (%) of productive tillers

(e) Height (cm)-from the base of the plant to tip of the uppermost fully expanded leaf.

(f) Flag leaf length (cm)

(g) Panicle length (cm)

(h) Filled grains panicle^{-1}

(i) Non-filled grains panicle^{-1}

(j) Ratio of filled grains to non-filled grains

(k) Percentage of grain filling

(l) Total number of grains panicle^{-1}

(m) Grain weight plant^{-1} (g plant^{-1})

(n) Panicle exsertion length (cm)

Figure 2. Wooden tub used in the greenhouse experiment to keep the pots flooded.

3.7 STATISTICAL ANALYSIS : All the data gathered were statistically analyzed using analysis of variance (ANOVA; JMP SAS software). The means were separated using Tukey's HSD test at an alpha level of 0.05. Chi-square tests were used to evaluate segregation ratios.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 PHENOTYPIC DESCRIPTION: All mutants exhibited an abnormal phenotype from an early stage of development. The most unique feature was the reduction of their stature and the presence of several tillers with fine culms forming a bushy phenotype. Figure 3 shows the phenotypic differences (height and tiller production) between the very high tillering and dwarf rice mutant lines and a normal rice plant at the same growth stage grown in the field. Table 1 shows the agronomic traits of the different mutant lines relative to Cocodrie and Zhe733 cultivars with the *sd-1* dwarfing gene. The mutant lines produced tillers faster than the conventional varieties. The first tiller of the mutant emerged two weeks after planting but Cocodrie and Zhe733 did not have any tillers. The two mutant lines (M-13662 & M-13684) produced more tillers than other mutant lines in our studies and the conventional cultivars. M-13684 produced the highest number of tillers (121.6) which was 7.8 times more than Cocodrie and 4.9 times than Zhe733. The other mutant, M-13662 had 5.5 times more tillers than Cocodrie and 3.5 times more than Zhe733 in field conditions. M-13684 had 1.36 times more tillers than M-13662. The lowest tiller count was obtained from Cocodrie but among the mutant lines, M-13621 had the lowest tiller count at 49.80. This tiller number was 3.19 times more than Cocodrie and twice that of Zhe733. M-13644, M-13660, M-13667 and M-13681 had more than 90% productive tillers which was similar to Cocodrie, but on average, the mutants had 84% productive tillers. Among the total tillers counted, Cocodrie had the highest percentage of productive tillers (93%) while the lowest was

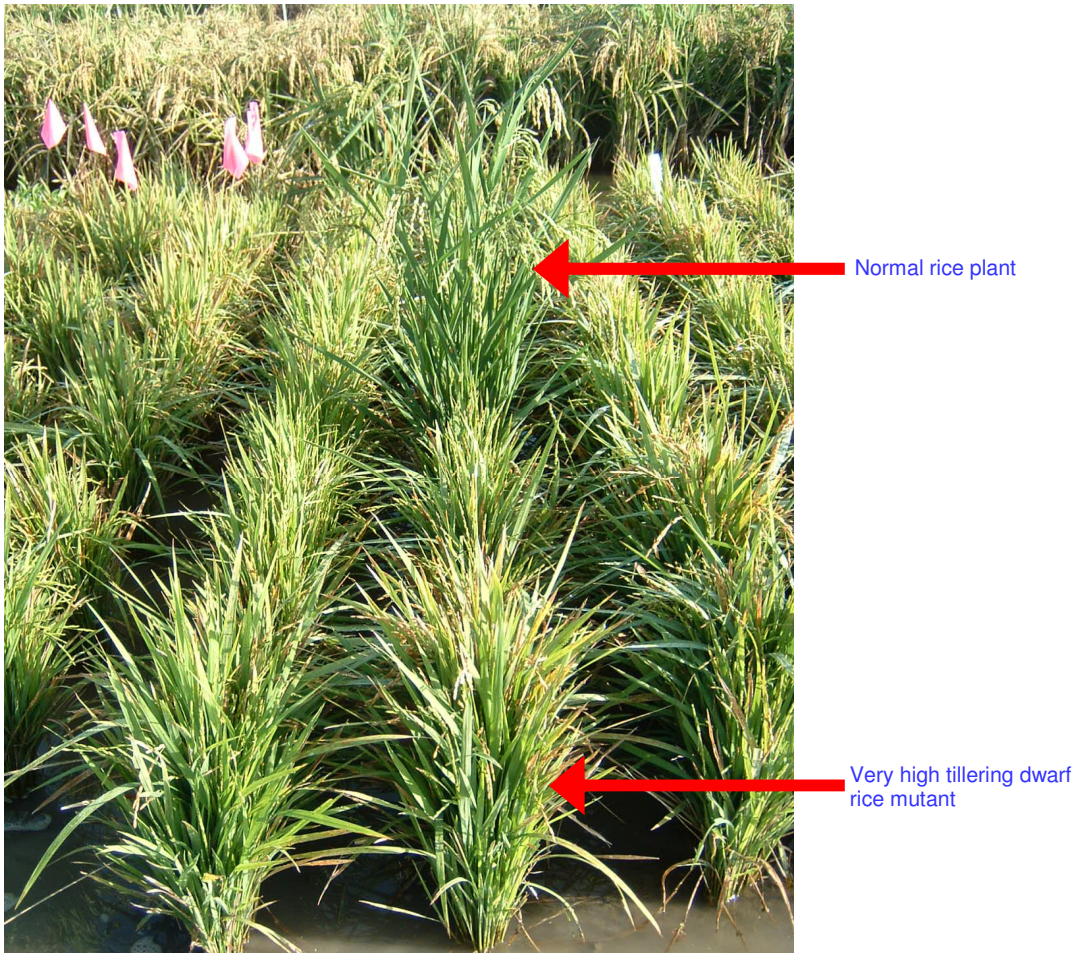


Figure 3. Phenotypic differences between very high tillering and dwarf rice mutant lines and normal rice plant.

Table 1. Agronomic traits of mutant lines and controls (Cocodrie & Zhe733) in field conditions.

Genotypes	Number of productive tillers plant^{-1}	Number of non-productive tillers plant^{-1}	Number of total tillers plant^{-1}	% of productive tillers	Height (cm)	Flag leaf length (cm)	Panicle length (cm)	Filled grains panicle^{-1}	Non-Filled grains panicle^{-1}
M-13603	57.00 ± 18.94	17.00 ± 3.16	74.00 ± 16.49	77.02	54.10 ± 6.06	21.89 ± 2.77	13.62 ± 1.48	16.28 ± 5.61	10.84 ± 6.67
M-13612	45.80 ± 4.86	15.80 ± 17.28	61.60 ± 20.45	74.35	55.37 ± 3.18	21.25 ± 3.00	12.95 ± 1.59	18.88 ± 7.52	7.16 ± 6.27
M-13621	41.00 ± 18.93	8.80 ± 5.97	49.80 ± 16.10	82.32	49.53 ± 2.24	17.16 ± 2.69	12.03 ± 2.25	19.08 ± 8.11	5.96 ± 6.43
M-13632	55.00 ± 14.56	16.80 ± 8.89	71.80 ± 13.46	76.60	47.49 ± 3.43	17.21 ± 2.48	12.39 ± 2.98	19.96 ± 8.97	8.48 ± 6.09
M-13640	55.60 ± 12.91	8.60 ± 6.50	64.20 ± 13.36	86.60	50.41 ± 3.90	20.29 ± 2.73	13.27 ± 1.48	23.36 ± 9.08	8.20 ± 6.40
M-13644	71.20 ± 8.40	7.40 ± 4.15	78.60 ± 9.09	90.58	54.35 ± 5.26	22.29 ± 3.37	14.09 ± 1.49	27.20 ± 8.80	13.44 ± 6.93
M-13652	51.80 ± 15.99	6.00 ± 3.67	57.80 ± 18.83	89.61	53.97 ± 2.28	19.89 ± 3.25	14.05 ± 1.76	27.00 ± 8.87	6.66 ± 4.07
M-13655	53.60 ± 24.86	9.60 ± 5.59	63.20 ± 25.62	84.81	50.67 ± 6.93	18.12 ± 5.07	12.35 ± 2.03	19.60 ± 10.30	8.68 ± 7.06
M-13660	56.60 ± 22.64	5.40 ± 3.13	62.00 ± 25.14	91.29	53.97 ± 3.73	19.40 ± 3.2	13.09 ± 1.50	23.08 ± 9.29	9.16 ± 7.72
M-13662	73.80 ± 26.45	15.20 ± 2.94	89.00 ± 26.30	82.92	50.67 ± 4.96	20.50 ± 2.69	11.68 ± 1.57	20.24 ± 8.00	7.72 ± 4.19
M-13667	68.00 ± 9.48	5.40 ± 0.89	73.40 ± 10.06	92.64	53.08 ± 1.65	22.68 ± 2.88	14.13 ± 1.19	22.36 ± 5.97	9.72 ± 3.54
M-13676	66.60 ± 11.17	9.40 ± 4.39	76.00 ± 14.94	87.63	51.68 ± 1.38	22.63 ± 2.65	14.26 ± 1.26	21.88 ± 6.73	11.44 ± 4.13
M-13681	73.40 ± 16.33	6.80 ± 3.11	80.20 ± 15.44	91.52	53.34 ± 2.00	23.00 ± 3.28	13.62 ± 1.13	22.92 ± 5.76	8.64 ± 3.93
M-13684	77.60 ± 23.58	44.00 ± 10.83	121.60 ± 24.29	63.81	52.83 ± 4.45	22.58 ± 3.39	12.35 ± 1.52	17.80 ± 6.40	10.32 ± 6.02
M-13689	63.40 ± 22.78	8.00 ± 3.31	71.40 ± 24.87	88.79	50.80 ± 5.95	22.48 ± 4.30	12.73 ± 2.07	19.68 ± 9.40	7.88 ± 4.80
M-13691	55.60 ± 17.12	10.08 ± 2.77	66.40 ± 19.75	83.73	54.10 ± 4.86	20.88 ± 4.05	12.49 ± 1.76	17.92 ± 7.07	8.84 ± 5.42
Cocodrie	14.60 ± 7.53	1.00 ± 0.70	15.60 ± 8.20	93.58	97.15 ± 2.10	25.38 ± 3.33	22.25 ± 1.75	123.56 ± 38.75	61.44 ± 20.14
Zhe733	17.60 ± 5.77	7.40 ± 3.13	25.00 ± 8.00	70.40	88.39 ± 4.63	27.83 ± 3.66	20.63 ± 1.13	87.52 ± 18.65	23.40 ± 13.18

† Measurements were taken in the form of mean ± SD, (Standard deviation), n=5.

Table 1. Continued.

Genotypes	Filled grains panicle^{-1}/Non-filled grains panicle^{-1}	% Grain filling	Number of total grains panicle^{-1}	Grain yield (g plant^{-1})	Panicle exsertion length (cm)	Heading days DAE*	Harvesting days DAE*	1000 grain weight (g)	Seed length (mm)	Seed width (mm)	Ratio (Seed length/Seed width)
M-13603	1.50	60.02	27.12 ± 10.26	14.01 ± 4.65	3.43 ± 1.57	93	121	15.10	7.12 ± 0.11	2.11 ± 0.22	3.37
M-13612	2.63	72.50	26.04 ± 9.00	13.57 ± 1.44	3.77 ± 2.40	93	121	15.70	7.46 ± 0.07	2.08 ± 0.15	3.58
M-13621	3.20	76.32	25.00 ± 11.52	11.89 ± 5.49	1.74 ± 1.64	92	121	15.20	7.70 ± 0.06	2.18 ± 0.11	3.53
M-13632	2.35	76.41	28.24 ± 10.99	16.41 ± 4.34	1.32 ± 1.43	93	121	14.95	7.86 ± 0.03	2.05 ± 0.15	3.83
M-13640	2.84	74.01	31.56 ± 10.82	20.26 ± 4.70	2.48 ± 1.64	92	121	15.60	7.99 ± 0.03	2.20 ± 0.08	3.63
M-13644	2.02	67.26	40.44 ± 11.62	29.82 ± 3.52	1.85 ± 1.29	93	121	15.40	8.10 ±0.04	2.11 ± 0.10	3.83
M-13652	3.86	76.48	33.66 ± 11.73	21.46 ± 6.62	2.33 ± 1.30	92	121	15.35	8.19 ± 0.02	2.09 ± 0.10	3.91
M-13655	2.27	69.30	28.28 ± 12.87	15.57 ± 7.22	2.32 ± 1.30	92	121	14.83	8.31 ±0.04	2.18 ± 0.13	3.81
M-13660	2.51	71.58	32.24 ± 11.84	19.56 ± 7.82	2.19 ± 1.58	92	121	14.98	8.58 ± 0.17	2.05 ± 0.18	3.91
M-13662	2.62	72.38	27.96 ± 9.61	24.09 ± 9.43	3.37 ± 1.77	95	121	15.50	7.87 ± 0.04	2.11 ± 0.12	3.72
M-13667	2.30	69.70	32.08 ± 7.25	23.82 ± 3.32	4.21 ± 2.02	92	121	15.67	7.47 ± 0.04	2.05 ± 0.09	3.64
M-13676	1.91	65.82	33.24 ± 7.86	22.58 ± 3.78	2.87 ± 1.67	92	121	15.50	7.60 ± 0.04	2.14 ± 0.08	3.55
M-13681	2.65	73.55	31.16 ± 6.84	27.90 ± 6.21	3.27 ± 2.23	95	121	16.59	7.76 ± 0.04	2.08 ± 0.11	3.74
M-13684	1.72	63.30	28.12 ± 10.47	21.40 ± 6.50	3.07 ± 1.58	93	121	15.50	7.85 ± 0.04	2.06 ± 0.16	3.81
M-13689	2.49	71.40	27.56 ± 10.95	19.62 ± 7.05	3.44 ± 1.77	92	121	15.73	7.95 ± 0.03	2.07 ± 0.15	3.84
M-13691	2.02	67.98	26.36 ± 9.08	15.22 ± 4.22	3.53 ± 1.63	92	121	14.85	8.07 ± 0.03	2.12 ± 0.11	3.80
Cocodrie	2.01	66.77	185.04 ± 45.33	45.35 ± 22.97	4.04 ± 1.88	75	105	24.80	8.91 ± 0.04	2.60 ± 0.05	3.15
Zhe733	3.74	79.18	110.52 ± 21.55	45.11 ± 14.79	4.70 ± 1.59	75	105	29.29	9.17 ±0.06	3.00 ± 0.11	3.05

† Measurements were taken in the form of mean ± SD (Standard deviation), n=5.
‡ * DAE – Days after emergence.

from M-13684 at 63%. It can be noted that the mutant lines had a narrow range for the number of productive tillers but differed widely in the number of non-productive tillers. The mutants had a tendency to produce small and fine tillers even at the maturity stage, thus increasing the total number of non-productive tillers. The 37% non-productive tillers of M-13684 were generally the newly emerged small tillers with 3-4 leaves at harvest. Based on the phenotype, the gene that promotes tiller growth in these mutants may be similar to *d3* in the five *d* mutants with weaker ability to suppress activity of tiller bud (Ishikawa et al., 2005) or rice *d10* with enhanced branching (Arite et al., 2007) indicating non-suppression of bud dormancy. The highest tiller count was 165 obtained in one of the M-13684 mutant plants at the maturity stage. This was more than the tiller count of the *htd-1* mutant with 99.5 ± 12.2 tillers (Zou et al., 2005) but less than nearly 200 tillers from *d10* (Arite et al., 2007). The reported height of *htd-1* mutant was nearly similar to Zhe733 with *sd-1* gene, thus our mutants resemble much with the d-phenotypes.

The weekly tiller count of mutants (M-13662 & M-13684) relative to controls is shown in Figure 4. Tiller number was doubled every week until seventh weeks after emergence of the first tiller in M-13662 as well as in M-13684 but the rate was lower in the controls (Cocodrie & Zhe733). The rates of production of new tillers in mutants slowed between the 13^{th} and 16^{th} week. However, after this period, the mutants produced tillers at a rate similar to that of the first seven weeks after emergence. The temporary cessation of new tiller production in mutants was observed for only three weeks

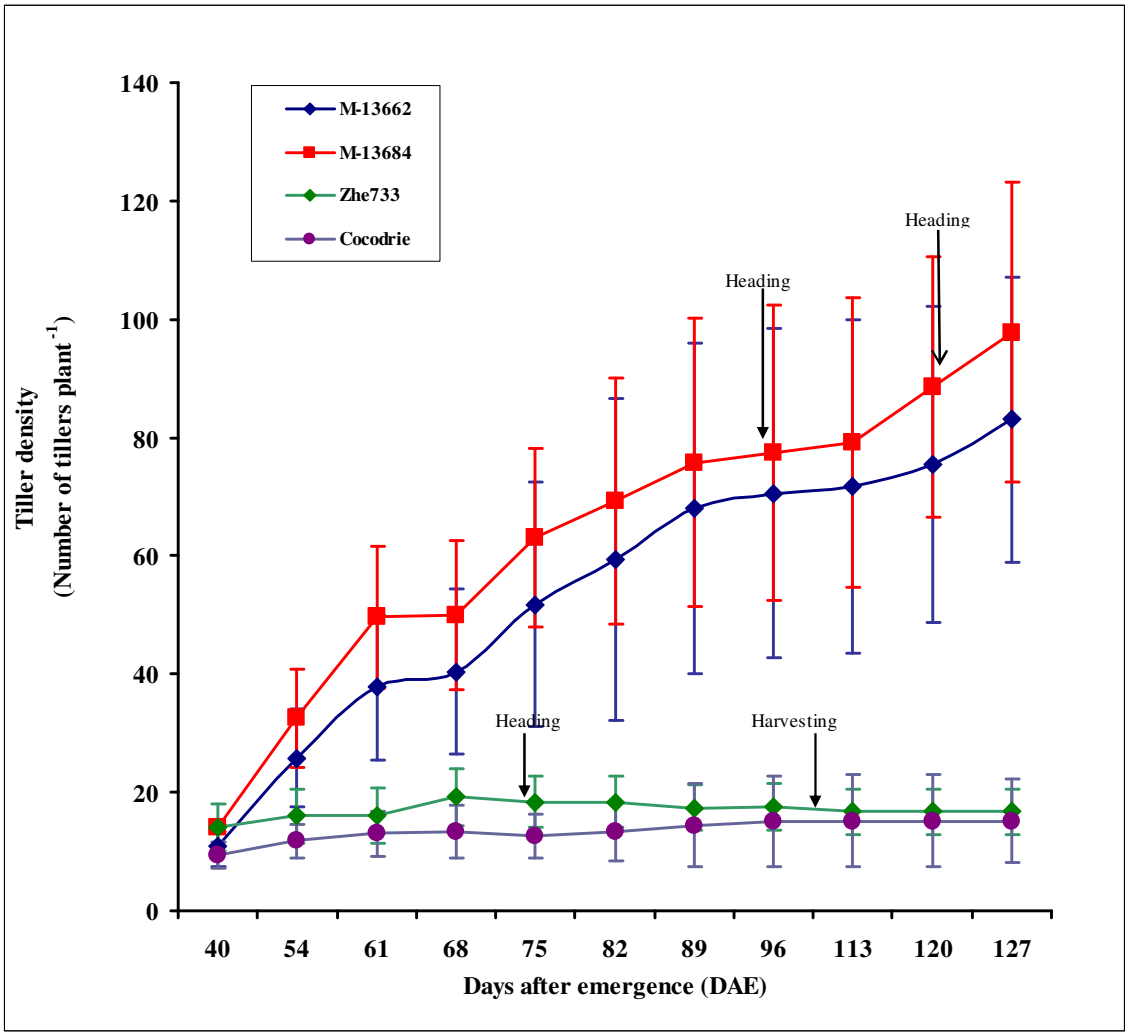


Figure 4. Weekly tiller production of field-grown very high tillering and dwarf rice mutants (M-13662 & M-13684) as compared to conventional cultivars (Zhe733 & Cocodrie) with semi-dwarf (*sd-1*) gene.

coinciding with the ripening of the grains (one week before, and two weeks after 50% flowering). Grains were generally ready for harvest 30 days after 50% flowering (heading date). The conventional rice nearly stopped producing tillers at one week before 50% flowering known as the maximum tillering stage. While the mutants started producing new tillers again two weeks after heading, a few tillers in the conventional rice started to dry and die. The late heading of the both mutants (M-13662 & M-13684) prolonged the period of tiller production; however, like Cocodrie and Zhe733, these mutants reached a maximum tiller count before heading. On average, the mutants were 36 and 45 cm shorter than Zhe733 and Cocodrie, respectively. M-13662 was the shortest (47.49 cm) and M-13612 (55.37) was the tallest among all mutant lines in the field condition. Based on the reported plant height, the mutants were close to the *d* mutants with height of about 40-65 cm (Arite al., 2007). The *htd-1* mutant was much taller at 83 cm (Zou et al., 2005) nearly as tall as Zhe733 with *sd-1* dwarfing gene. The average height of the rice mutants (M-13662 & M-13684) was only 60-64% of the recently identified high tillering dwarf rice mutant, *htd-1*. The different growing conditions could be the main cause of these differences, thus a valid comparison can only be made if these mutants are grown together at the same time and location. Development and evaluation of isolines of the mutants will further verify their phenotype similarities or differences. Dwarfness of the M-13662 and M-13684 mutants could be attributed to the abnormal pattern of internode elongation and these patterns were observed for the other mutant lines. The mutants headed after 95 days of planting and it took 121 days to ripen whereas conventional rice headed after 75 days of planting and it took 105 days to ripen. The

average flag leaf length of mutants was 21.50 cm. The longest and shortest flag leaf lengths were from Zhe733 (27.83 cm) and M-13621 (17.16 cm), respectively. Among the mutants, the longest flag leaf length (23 cm) was observed in M-13681. The flag leaf length of M-13662 (20.50 cm) was shorter than M-13684 (22.58 cm). It was also observed that the mutants had darker green leaves at maturity than Cocodrie and Zhe733. The mutants had much shorter panicles than Cocodrie and Zhe733 as shown in Figure 5. The mutants had an average panicle length of 13.94 cm. M-13662 had shorter panicle at 11.68 cm than M-13684 with 12.35 cm but both had almost the same number of grains panicle^{-1} (28-29 grains panicle^{-1}). The mutants needed only 26 days for grain filling because of the small number of grains panicle^{-1}. The longest and the shortest mutant panicles were from M-13676 (14.26 cm) and M-13632 (11.45 cm), respectively. On the other hand, the panicle lengths of the controls (Cocodrie & Zhe733) were more than two times longer than the panicle lengths of the rice mutants (M-13662 & M-13684). The highest (185.04) and lowest (25.00) number of grains panicle^{-1} were from Cocodrie and M-13621, respectively.

The pattern of flowering was normal for all entries and the mutants had an average panicle exsertion length of 2.90 cm. Cocodrie and Zhe733 had 4.04 cm and 4.70 cm panicle exsertion length respectively and these were well exserted as compared to other mutants in our studies, Among the mutants entries, M-13667 had the most exserted panicle (4.21 cm) while the least was M-13632 (1.32 cm). M-13662 had a longer exsertion length (3.37 cm) as compared to M-13684 (3.07 cm). The percentage of grain filling varied for all entries. The highest and lowest grain filling was obtained from

Figure 5. Comparison of panicle length and number of grains panicle^{-1} between very high tillering and dwarf rice mutants (M-13662 & M-13684) and controls (Cocodrie & Zhe733).

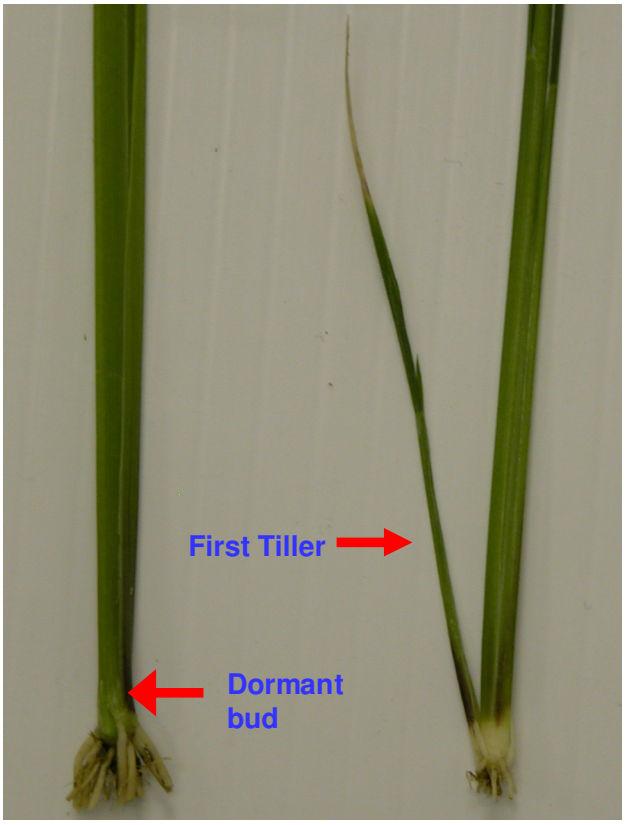
Zhe733 and M-13684, respectively. Among the mutants, the highest grain filling was observed in M-13652. Generally, the growth of the rice plant is completed during the ripening stage and photosynthates are accumulated in panicles. Apart from accumulation of photosynthates in panicles (sink) resulting from flag leaf photosynthesis, mobile carbohydrates, protein, and mineral nutrients, from different sources also move to the panicles during the grain filling stage and the plant gradually becomes senescent (Murayama, 1995). The number of grains in a panicle was very low in the mutant as compared to controls (Cocodrie & Zhe733). It is likely that the mobile carbohydrates, protein, and mineral nutrients from different sources were not transported to the panicles and these were used to produce large number of tillers even after the harvesting stage. The mutant lines also produced smaller seeds relative to controls. The length and width of the seeds of both mutants (M-13662 & M-13684) were less than Cocodrie and Zhe733. Considering the ratio of seed length and width, both mutants (M-13662: 3.72 & M-13684: 3.81) were nearly comparable to the controls (Cocodrie= 3.42 & Zhe733= 3.05). With these ratios, all entries can be grouped under the long grain category. Rice in the U.S. is generally grouped as long (> 3.0:1), medium (2.0:1) and short grain (<2.0:1). The highest 1000 seed weight was obtained from Zhe733 (29.29 gm) followed by Cocodrie (24.80 gm). The highest and lowest 1000 seed weight among all mutant lines were obtained from M-13681 (16.59 gm) and M-13655 (14.83 gm), respectively. The seed weight indicated the smaller seeds of mutants relative to Cocodrie and Zhe733.

Takeda et al. (2003) described the *fc1* mutant as showing a dwarf phenotype and increased branching, and recently, five rice tillering dwarf mutant genes (*D3, D10, D14,*

D17 and *D27*) and a high tillering dwarf 1, (*htd-1*), were reported and characterized. These mutants showed reduced plant height and increased tiller number (Ishikawa et al., 2005; Zou et al., 2005). The very high tillering dwarf rice mutants (M-13662 & M-13684) in this study have phenotypes similar to the previously described rice mutants (increased tiller number and dwarfness) but have the unique trait of continuous tiller production. The *d* mutants, *htd-1*, *fc1,* M-13662 and M-13684 can be differentiated from each other on the basis of dwarfing pattern. Arite et al. (2007) reported that elongation was suppressed in all internodes in *d* mutants, which accounts for their dwarf phenotype, whereas shortening of the most apical internode was observed in *fc1*. Average shortening of the top four internodes and panicle is the main cause of dwarfness in *htd-1* mutant. M-13662 and M-13684 was like *htd-1* in the internode elongation pattern of the top four internodes but compression of 2-3 basal internodes also accounted for the dwarfness of both mutant (M-13662 & M-13684) in addition to average shortening of top internodes. The tremendous tiller production might account for the shorter stature of both mutants as compared to the *htd-1* mutant. From the early stage of development, M-13662 and M-13684 showed a similar phenotype; a tremendous increase in tiller numbers, a reduction of plant stature and small grain size and these were similar to the *d* mutants (*d3, d10, d14, d1,* and *d27*). Dwarf and high tillering traits were associated traits in all reported high tillering dwarf mutants of rice (Zou et al., 2005) similar to branching and dwarfism in Arabidopsis and pea (Sorefan et al., 2003; Goto et al., 2005). The same associated traits were observed in our rice mutants (M-13662 & M-13684). The short stature of the

mutant could be the result of reduced apical dominance, however, it should be determined in future studies.

Destructive sampling showed the developmental pattern of tiller and leaf growth in the very high tillering and dwarf rice mutant. The first fully expanded leaf of the main culm was formed approximately 9 days after planting in mutants and each new fully expanded leaf was formed every 4 days. Almost the same pattern of leaf formation was observed for control (Cocodrie). The lateral bud under the first leaf sheath remains dormant in wild type japonica cultivars (Cocodrie), whereas this bud was active in both rice mutants (M-13662 & M-13684). The first clear evidence of a difference between mutants and control was the out-growth of tiller buds in the first leaf axil at the 3rd leaf stage in the mutants, which was not observed in the wild type plants (Cocodrie). The first tiller with a fully expanded leaf was observed at the 4th leaf stage in case of both mutant lines (M-13662 & M-13684), whereas, no tiller was observed in Cocodrie (Figure 6). On average, mutants produced 2-3 tillers at the 5th leaf stage but no tillers were formed in Cocodrie even at the 5th leaf stage. Both mutants produced eight times (24) the tillers of Cocodrie (3) 48 days after seedling emergence. The average height of both mutants becomes almost static 48-52 days after seedling emergence but an increase in height was observed in Cocodrie after 48-52 days. At the 6th leaf stage, the M-13684 had four emerged tillers (two primary and two secondary) while Cocodrie had only one primary tiller (Figure 7). There was only one axillary bud present in the leaf axils of the rice mutants (M-13662 & M-13684) similar to the control plants (Cocodrie & Zhe733).



Cocodrie Mutant

Figure 6. Emergence of 1st tiller at 4th leaf stage.

Cocodrie M-13662 M-13684

Figure 7. Comparison of tiller production rate at the 6th leaf stage of Cocodrie and the two very high tillering and dwarf rice mutants (M-13662 & M-13684).

On average, the mother culm of the mutant produced 3-4 primary tillers while each of the primary tillers produced 2-3 secondary tillers. Each secondary tiller was able to produce 1-2 tertiary tiller and tertiary tiller further produced 1-2 quaternary tillers and quaternary tillers produced at least one tiller and so on. In the wild type (Cocodrie and Zhe733), however, only the main culm, primary tiller and secondary tillers produced new tillers. The pattern of tillering in the mutant is shown in Figure 8. It was also observed that the first 2-3 nodes of the mutants (M-13662 & M-13684) were so compressed forming a structure like a crown thus it was difficult to differentiate between these nodes by the naked eye during destructive sampling. This could be another reason for the dwarfness of the mutants in addition to the shortening of the top four internodes. The top four internodes of the M-13662, M-13684 and Cocodrie were measured and compared. Based on the dwarfing pattern of the top four internodes, the very high tillering and dwarf rice mutants could be categorized into the dn-type dwarf defined by Takeda, but a few plants had different dwarfing patterns not included in the same classification. Second and fourth internodes were found shorter than third internodes in few plants of M-13662 & M-13684. Likewise, third internode was shorter than second and fourth internode in some other plants of both mutants (M-13662 & M-13684) (Figure 9).

Usually, wild type cultivars (Cocodrie & Zhe733) increase their tiller numbers until the onset of culm elongation and panicle initiation, and then the tiller numbers of each plant are static or started decreasing because of the senescence of old tillers.

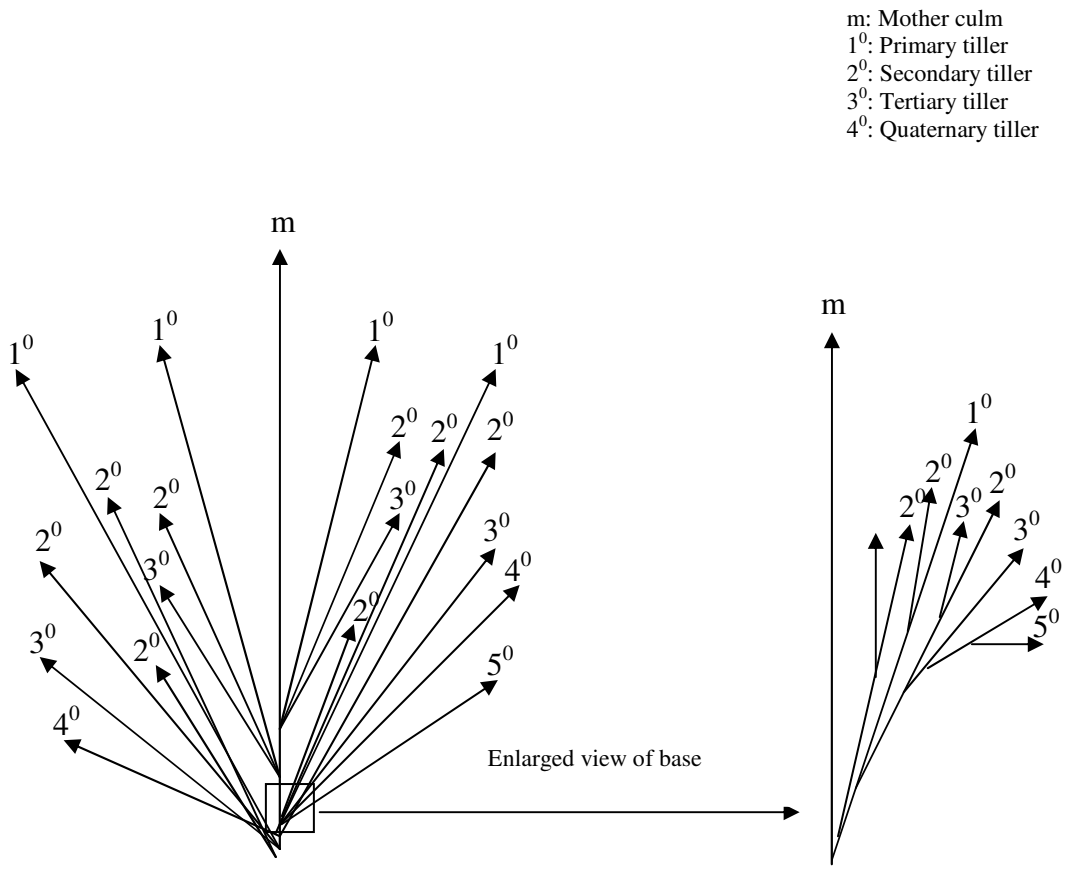


Figure 8. Schematic diagram of tiller formation in the very high tillering and dwarf rice mutants (M-13662 & M-13684).

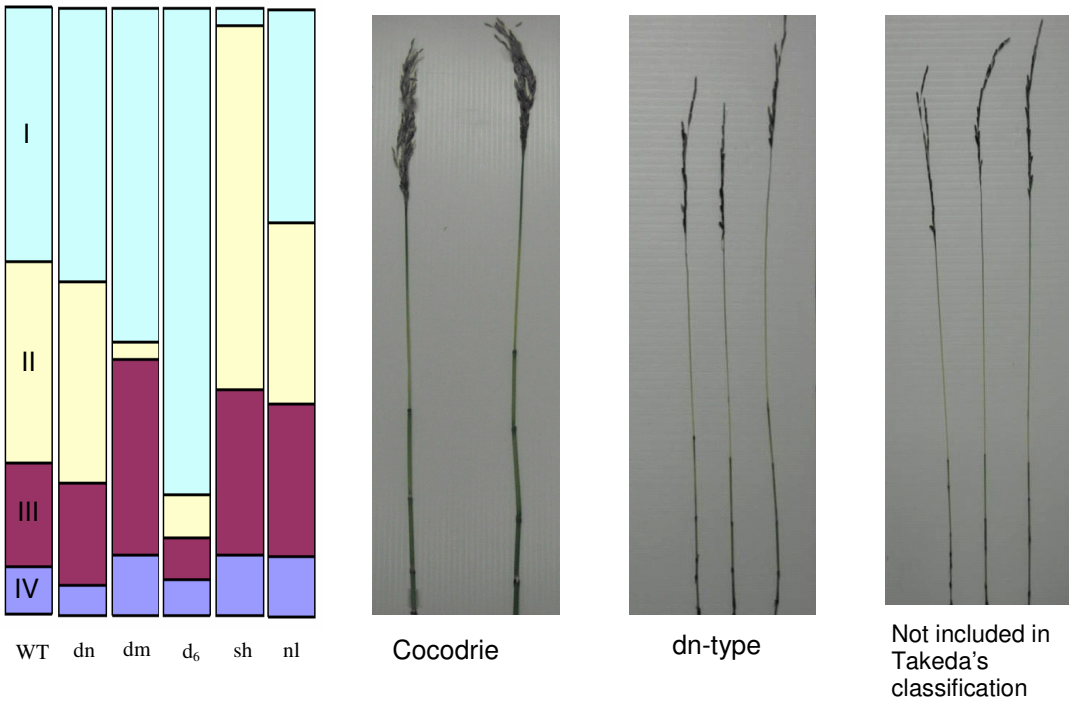


Figure 9. Elongation pattern of the top four internodes in wild type and the very high tillering and dwarf rice mutants (M-13662 & M-13684). (a) Schematic representation of internode elongation patterns of wild type (WT) and various rice dwarf mutants (d6-, dn-, dm-, nl- and sh- types; redrawn from Takeda, 1977). (b) Internode elongation pattern of Cocodrie, showing dn-type pattern. (c) Very high tillering and dwarf rice mutant lines showing dn-type internode elongation pattern. (d) Few plants of M-13662 and M-13684 showing the unique internode elongation patterns that were not included in Takeda's classification.

However, the very high tillering and dwarf rice mutant lines (M-13662 & M-13684) kept producing new tillers even at the harvesting stage. The same number of tillers and height were observed in both destructive sampling and field phenotyping until 48-52 days after seedling emergence indicating consistent trait expression at different growing environment. These observations further suggest that the enhanced tillering capacity of the very high tillering and dwarf mutant lines is the result of the release of axillary buds from their dormant state. The same observation was made in *htd-1*, *d3* and *d10* rice mutants (Ishikawa et al., 2005; Zou et al., 2005; Arite et al., 2007). Both mutants (M-13662 & M-13684) described here never stops producing new tillers even at the late reproductive stage. Each mutant tiller (main culm, primary tiller, secondary tiller, tertiary tiller, quaternary tiller and beyond quaternary tiller) had the capacity to serve as a tiller source for further tiller production, whereas only the main culm, primary and secondary tillers of wild type had the same capacity to serve as mother tillers and produces new tillers. High tillering trait is important in biomass production as tillers accounted for 75% of the biomass in rice (Wu et al., 1998) and the efficiency of ratoon crops also depends upon the rate of tiller production after harvesting. Thus, these mutants might be a good material for higher biomass production and ratooning studies.

4.2 THE RESPONSE OF THE VERY HIGH TILLERING AND DWARF RICE MUTANTS (M-13662 & M-13684) TO GIBBERELLIC ACID: An examination of the elongation of second leaf sheath of the mutant as well as control in response to GA application (200 pmol) was conducted to determine the possible relation between the dwarfing of the mutants (M-13662 & M-13684) and its ability to respond to GA

application. Even before GA application, Cocodrie had a longer 2nd leaf sheath than the mutants and it had the longest leaf sheath among the entries. M-13684 had the shortest leaf sheath among all genotypes (Table 2). M-13662 and M-13684 had just a 0.94 cm and 0.79 cm increase in the length of the leaf sheath after application of GA_3, respectively and Cocodrie and M-13662 had a 0.99 cm increase but the test of means using t-test supported the differences. Considering the ratios, all three lines gave approximately the same ratios of the lengths of the second leaf sheath with and without application of GA. It indicates that both mutants (M-13662 & M-13684) were GA-responsive, similar to Cocodrie. Cocodrie is a semi-dwarf rice cultivar with the *sd-1*, gene and *sd-1* plants were shown to retained responsiveness to GA at seedling, tillering and heading stage (He and Li, 1996; Mitsunaga et al., 1994) supporting the above observations. Spielmeyer et al. (2002) proposed that *Os20ox2* gene correspond to *sd-1* locus and the semi-dwarf phenotype was due to defective *Os20ox2* gene, resulting to deficiency of active GA. It was shown that GA_3, the substrate of GA20-oxidase was accumulating in elongating stems of semi-dwarf plants but the content of major product (GA_{20}) and bioactive GA_1 were low relative to tall phenotypes. The *sd-1* gene cloned in Japan (Mona et al., 2002) revealed to encode the same GA20-oxidase and the lower amounts of GA_{20} and bioactive GA_1 in semi-dwarf cultivars causes the short phenotype. Both rice mutants (M-13662 & M-13684) retained the ability to respond to the applications of bioactive gibberellins similar to Cocodrie, the difference between the mutants (M-13662 & M-13684) and Cocodrie (*sd-1*) could be in the amount of endogenous bioactive GA_1. It is possible that the mutants had much less bioactive GA_1

than Cocodrie, thereby reducing the plant height. Two independent alleles of *sd-1* gene had different amount of GA_1, about 20-35% less than tall plants. These reductions in GA, however, were not substantiate enough to remarkably reduce height in the semi-dwarf plants.

Reduction of plant height is another unique agronomic trait/characteristic of the mutants besides the tremendous number of tiller produced. Gibberellic acid has a very important role in controlling rice plant height and has equal importance in the growth of rice leaf sheaths (Matsukara et al., 1998; Yamamuro et al., 2000; Sasaki et al., 2002; Wang and Li, 2005). Application of GA_3 to Tan-ginbozu, a GA-deficient dwarf mutant of rice, restored the normal phenotype (Murakami, 1968). The mutants (M-13662 & M-13684) may be GA deficient mutants based on phenotype. Sakamoto et al. (2004) screened for GA deficient mutants using the following criteria: dwarfism without other aberrant morphology, dark green leaves and restoration of dwarfism to wild type by GA_3 treatment. Although the leaves of the mutants (M-13662 & M-13684) were not dark green and the phenotype was not restored to normal with GA_3 application, it had abnormal morphology other than being short and responded to GA_3 like *sd-1* plant known to be GA deficient (Spielmeyer et al., 2002). Quantitative analysis of endogenous GA levels is needed to elucidate its possible role in the dwarfness of M-13662 & M-13684.

Table 2. Effect of gibberellic acid (GA) on the elongation of the second leaf sheath. Mean ± SD, n=10.

Variety	Length of the second leaf sheath (cm). Mean ± SD, n=10		Ratio
	$-GA_3$	$+GA_3$	$+GA_3/-GA_3$
Cocodrie	5.18 ± 0.62	6.17 ± 0.58	1.19
M-13662	4.69 ± 0.66	5.63 ± 0.91	1.20
M-13684	3.41 ± 0.65	4.20 ± 0.65	1.23

† GA_3, Gibberellic acid; - = without GA_3; + = with GA_3
‡ SD, Standard deviation

4.3 GENETIC ANALYSIS: The ten F_1 plants from the three crosses between M-13662 & M-13684 and Cocodrie showed a wild type phenotype similar to Cocodrie, which is typical for a recessive trait. Only two distinct phenotypes were observed in the 1,246 individuals from three F_2 populations grown in the greenhouse, thus the plants were classified into two groups according to these phenotypes. The tall plants with few tillers like Cocodrie dominated the population and the dwarf plants with a high number of tillers like the mutant were less in number. After counting the plants in each group, it was determined that the F_2 progenies segregated in approximately a 3: 1 ratio (3 wild type to 1 dwarf and high tillering mutant type). Chi-square analysis ($X^2<X^2_{0.05}$) showed a fit between observed and expected ratios (Table 3). Based on the segregation analysis, it was concluded that the very high tillering and dwarf traits of the rice mutants were controlled by a single recessive gene.

No plants in these populations were much taller than Cocodrie and none were much shorter than the mutant. Cocodrie is known to have a recessive semi-dwarf gene,

sd-1. If the two genes are allelic or closely linked, there should be no segregation and if these were two independent genes, at least four classes should be identified. The 3:1 ratio suggests that Cocodrie has the wild dominant allele that was absent in the mutant lines. Analysis of SSR markers linked to *sd-1* indicated that both mutants had *sd-1* gene and these were derived from the parents (L-202 & Saber), both having *sd-1* gene. The gene controlling the traits in the mutant lines could be another allele at *sd-1*allele locus acting recessive to *sd-1* in Cocodrie or it involves another locus. Spielmeyer et al. (2002) reported two independent alleles at *sd-1* locus (*Os20ox*), GA20-*oxidase*. Both had alteration with *Os20ox* (a deletion of 280 bp and amino acid substitution) resulting to deficiency of active GA. The same *sd-1* locus was reported by Mona et al., (2001) but existence of at least one more locus of GA20-*oxidase* was suggested. Screening candidates genes and evaluating GA-deficient mutants, Sakamoto et al. (2004) reported four *GA20ox* like genes located in chromosome 1, 7, 5, and 7. The gene located in chromosome 1 was the previously reported *sd-1* gene.

Table 3. Chi-square analysis of the segregation ratios of F_2 population derived from mutant and Cocodrie cross.

Cross	Wild type (Cocodrie)	Mutant	Total	X^2 (3:1)
M-13662*/Cocodrie	281	86	367	0.48
M/13684*/Cocodrie	313	100	413	0.13
Cocodrie/M-13662*	336	130	466	2.08

† X^2: Chi square
* Mutant

4.4 RESPONSE OF THE VERY HIGH TILLERING AND DWARF RICE MUTANTS (M-13662 & M-13684), COCODRIE AND ZHE733 TO VARYING LEVELS OF NITROGEN AND POPULATION DENSITIES: The analysis of variance (ANOVA) for traits measured in the greenhouse experiment is shown in Table 4. The variation due to genotype was highly significant for all agronomic traits evaluated and there were no significant differences observed among the three different nitrogen levels for any agronomic traits studied except panicle length. The longest panicle was observed at the highest nitrogen level and the shortest was from the lowest nitrogen level. The density and genotype x density interaction were significant for all agronomic traits studied except panicle exsertion length. A genotype x nitrogen level interaction was found significant for the number of non-productive tillers, total number of tillers and total grains panicle^{-1}. The ANOVA also indicated that the genotype x density x nitrogen interaction was not significant for all studied agronomic traits.

Comparisons among the means of various traits of the genotypes across N level and densities indicated significant differences among entries (Table 5). M-13662 and M-13684 had statistically the same number of productive and non-productive tillers, total tillers, plant-height, flag-leaf length, panicle length, filled-grain panicle^{-1}, non-filled grain panicle^{-1}, total grains panicle^{-1}, grain yield plant^{-1} and panicle exsertion length. The very high tillering and dwarf rice mutants (M-13662 & M-13684) were significantly different from conventional rice (Cocodrie & Zhe733) for productive tiller number, non-productive tiller number, total tiller number, plant height, flag leaf length, panicle length,

Table 4. Means squares of the ANOVA showing the effects of N-levels, genotype, population density and their interaction on number of productive tillers, number of non-productive tillers, number of total tillers, plant height, flag leaf length, panicle length, filled grains panicle^{-1}, non-filled grains panicle^{-1}, total grains panicle^{-1}, grain yield plant^{-1} and panicle exsertion length.

		Number of productive tillers		Number of non-productive tillers		Number of total tillers		Plant height		Flag leaf length	
Source	df	MSE	Prob > F	MSE	Prob > F	MSE	Prob > F	MSE	Prob > F	MSE	Prob > F
Rep(N-level)	6	93.203	NS	74.0787	**	253.701	NS	20.8167	NS	24.1154	*
N-level	2	200.917	NS	139.973	NS	628.047	NS	12.2056	NS	13.4273	NS
genotype	3	21599	**	966.863	**	31682.3	**	30586.2	**	613.551	**
density	4	7479.88	**	448.658	**	11574.6	**	439.689	**	102.096	**
genotype*density	12	2001.39	**	105.344	**	3008.87	**	111.219	**	33.7122	**
genotype*N-level	6	150.438	NS	94.7489	**	386.064	**	37.6426	NS	23.2194	NS
density*N-level	8	79.353	NS	23.3215	NS	141.249	NS	20.4972	NS	12.0848	NS
genotype*density*N-level	24	72.5186	NS	17.8717	NS	92.2979	NS	12.5824	NS	7.60969	NS

† *, ** Significant at the 5 and 1% levels of probability, respectively. NS = Not significant at the 5% level of probability.
‡ N-level: Nitrogen level. g: Gram

Table 4. (continued).

		Panicle length		Filled grains panicle^{-1}		Non-filled grains panicle^{-1}		Total grains panicle^{-1}		Grain yield (g plant^{-1})		Panicle exsertion Length	
Source	df	MSE	Prob > F	MSE	Prob > F	MSE	Prob > F	MSE	Prob > F	MSE	Prob > F	MSE	Prob > F
Rep(N-level)	6	1.55188	NS	444.374	**	367.365	NS	1172.13	*	23.1672	NS	3.19368	NS
N-level	2	9.58952	*	683.17	NS	338.203	NS	2367.16	NS	48.0291	NS	5.65291	NS
genotype	3	1153.23	**	57173.8	**	30224.3	**	120868	**	182.679	**	142.326	**
density	4	23.9009	**	1181.4	**	3861.43	**	4024.09	**	1104.78	**	3.49835	NS
genotype*density	12	7.20718	**	363.115	**	2030.09	**	1218.07	**	49.4341	**	5.10321	NS
genotype*N-level	6	2.95	NS	188.058	NS	296.621	NS	1027.19	*	23.8629	NS	9.10115	NS
density*N-level	8	2.19042	NS	169.665	NS	163.685	NS	510.402	NS	15.9108	NS	2.68604	NS
genotype*density*N-level	24	1.82405	NS	180.958	NS	281.603	NS	224.932	NS	13.3422	NS	4.83856	NS

† *, ** Significant at the 5 and 1% levels of probability, respectively. NS = Not significant at the 5% level of probability.
‡ N-level: Nitrogen level. g: Gram

Table 5. Means of agronomic traits of the very high tillering and dwarf rice mutants (M-13662 & M-13684), Cocodrie and Zhe733 across three levels of nitrogen and five different plant densities.

Genotypes	Number of productive tillers	Number of non-productive tillers	Number of total tillers	% of productive tillers	Plant height (cm)	Flag leaf length (cm)	Panicle length (cm)	Filled grains panicle^{-1}	Non-filled grains panicle^{-1}	Total grains panicle^{-1}	Grain yield (g plant^{-1})	Panicle exsertion length (cm)
M-13662	39.95a	7.93a	47.88a	83.43	63.51c	25.81c	12.48c	17.19c	13.29c	30.48b	10.32a	1.73b
M-13684	42.36a	9.15a	51.52a	82.22	63.08c	25.42c	12.60c	11.53c	17.94c	29.48b	8.18ab	1.94b
Cocodrie	2.61b	0.23b	2.85b	91.57	103.15b	29.11b	20.18b	88.57a	51.96b	124.66a	5.87b	4.55a
Zhe733	3.90b	0.91b	4.82b	80.91	112.73a	33.38a	22.20a	52.00b	66.43a	114.22a	6.38b	5.21a

† Within columns, means followed by a common lowercase letters are not significantly different at the 0.05 level.
‡ g: Gram.

filled grains panicle^{-1}, non-filled grains panicle^{-1}, total grains panicle^{-1} and panicle exsertion length. Both mutant lines produced more tillers than conventional cultivars. M-13684 and Cocodrie produced the highest (51.52) and the lowest tiller (2.85) number, respectively across three levels of nitrogen and five plant densities. The same trend was observed in field conditions but their values were much lower in the greenhouse. The final tiller count of both mutants was least affected relative to controls in greenhouse condition. A drastic reduction of final tiller count of Cocodrie was observed in the greenhouse as compared to the field. M-13662 and M-13684 produced 53.79% and 42.36%, respectively of the total field tiller count in the greenhouse but Cocodrie and Zhe733 produced only 18.26% and 19.28% of the total field tiller count, respectively. Although M-13662 had many more tillers than Cocodrie and Zhe733, it followed the trend of having lower tiller counts in the greenhouse compared to the field planting like the two conventional cultivars. Among the total tillers counted, Cocodrie had the highest percentage of productive tillers (91.57%) while the lowest was from Zhe733 at 80.91%. The percentage of productive tillers of both mutants was higher than Zhe733 but lower than Cocodrie. The percentage of productive tillers of Cocodrie and M-13662 was nearly the same for both field and greenhouse conditions, but the proportion of productive tillers was increased under varying levels of nitrogen and plant densities for M-13684 and Zhe733. The height of the mutants and Zhe733 differed by 36-38 cm and 45-47 cm relative to Cocodrie in the greenhouse, but under field conditions these varied from 40 to 49 cm. Plants were 25%, 28% and 6% taller in the greenhouse compared to field conditions for the mutants, Zhe733 and Cocodrie, respectively, across different nitrogen

fertilization and plant densities. The increase in plant height in the greenhouse might be due to less competition and more favorable environmental conditions. The flag leaf length of the mutants (M-13662 & M-13684) was approximately 12-23% shorter than the controls (Cocodrie & Zhe733). Similarly, the panicle length of the mutant lines was 55-60% shorter than the controls but the increase in flag leaf length was observed in the greenhouse as compared to field conditions for both mutants as well as controls. M-13662 mutant was significantly different from both controls (Cocodrie & Zhe733) with highest grain yield plant^{-1} (10.32 g) whereas, lowest grain yield plant^{-1} was recorded from Cocodrie cultivar (5.87 g). M-13684 mutant had statistically same grain yield plant^{-1} as Cocodrie and Zhe733 cultivars. Although the mutant had few grains panicle^{-1}, the large number of tillers compensated for this, thereby increasing grain yield plant^{-1} in greenhouse condition. In contrast, controls (Cocodrie & Zhe733) produced highest grain yield plant^{-1} in field condition as compared to both mutants (M-13662 & M-13684). This can be attributed to more productive tillers and grains panicle^{-1} in the controls at field condition. The two mutant lines however, were consistent for grain yield plant-^{1} in both growing conditions (greenhouse and field study). Tillering ability of rice plant is known to impact panicle production and it was highly correlated with grain yield (Counce and Wells, 1990; Miller et al., 1991). Significant differences for plant-height, flag leaf length, panicle length, filled grains panicle^{-1}, and non-filled grains panicle^{-1} were observed between Cocodrie and Zhe733, but not for productive tiller number, non-productive tiller number, total tiller number, total grains panicle^{-1}, grain yield plant^{-1} and panicle exsertion length.

Across genotypes and nitrogen levels, the highest number of productive, non-productive and total tillers was obtained at one plant per pot and it decreased as planting density increased. Increased numbers of productive tillers at lower plant density was also reported in previous studies (Ottis and Talbert, 2005). A reduction of plant height, flag-leaf length, panicle length, filled grains panicle^{-1}, non-filled grains panicle^{-1}, total grains panicle^{-1}, and grain yield plant^{-1} was observed at higher plant density compared to lower plant density (Table 6). There was no significant difference between one and two plants pot^{-1} for plant height, flag leaf length, panicle length, filled grains panicle^{-1} and total grains panicle^{-1} across genotypes and nitrogen levels. Higher numbers of non-filled grains panicle^{-1} were observed at one plant pot^{-1} than at two plants pot^{-1}. No other significant differences were observed among three, four and five plants pot^{-1} for plant height, flag leaf length, panicle length, filled grains panicle^{-1}, non-filled grains panicle^{-1}, total grains panicle^{-1} and grain yield panicle^{-1}. This can be attributed to higher competition among plants at higher plant densities.

A comparison of means across N levels reflecting genotype x density interactions is shown in Table 7. The highest number of productive, non-productive and total tillers was found in both mutants at the lowest density and it decreased as planting density increased. Although the controls had statistically the same number of productive tillers, non-productive tillers and total tillers, the trend of decreasing values for each trait was also observed (Table 7). These results suggest that the tillering ability of both mutants is affected by different levels of plant density. The same response of high tillering mutants to planting density was reported by Ishikawa et al., (2005). Even

the highest tillering mutant (*Id3*) responded to planting density. The different levels of planting densities had no effect on the plant height, flag leaf length, panicle length, filled grains panicle^{-1}, non-filled grains panicle^{-1} and total grains panicle^{-1} for both mutants but this was not the case for the control (Table 7). Different plant densities had significant effect on grain yield plant^{-1} in both mutants (M-13662 & M-13684) and Zhe733 cultivar whereas Cocodrie cultivar yielded same at different plant densities. Highest grain yield plant^{-1}was achieved at lowest plant density in M-13662 among four genotypes. The highest grain yield plant^{-1} of M-13662 is the result of higher percentage of productive tillers as well as higher tiller number. A reduction in plant height, flag leaf length, panicle length, number of filled grains panicle^{-1}, number of non-filled grains panicle^{-1} and number of total grains panicle^{-1} was observed at higher planting densities in Cocodrie. Previous research has also reported that as rice seeding rate increased, panicle density increased but filled grains panicle^{-1} decreased with no changes in yield (Jones and Synder, 1987; Gravois and Helms, 1992).

Table 6. Effects of plant densities to mean agronomic traits across four genotypes and three N levels.

Density level	Number of productive tillers	Number of non-productive tillers	Number of total tillers	% of productive tillers	Plant height (cm)	Flag leaf length (cm)	Panicle length (cm)	Filled grains panicle^{-1}	Non-filled grains panicle^{-1}	Total grains panicle^{-1}	Grain yield (g plant^{-1})
D1	46.72a	10.61a	57.33a	81.49	89.61a	30.98a	17.88a	50.95a	52.79a	86.34a	16.85a
D2	22.40b	4.18b	26.58b	84.27	89.02a	29.10ab	17.59a	45.47ab	43.23b	86.11a	8.70b
D3	17.63bc	3.91b	21.55bc	81.80	84.52b	27.81bc	16.52b	38.14b	31.91c	66.78b	5.64c
D4	13.94cd	2.27b	16.22cd	85.94	82.86b	26.56c	16.11b	39.03b	31.08c	68.72b	4.14c
D5	10.33d	1.82b	12.16d	84.95	82.08b	27.71bc	16.22b	38.01b	28.01c	65.59b	3.10c

† Within columns, means followed by a common lowercase letters are not significantly different at the 0.05 level.
‡ D1= 1 plant pot^{-1}, D2= 2 plants pot^{-1}, D3= 3 plants pot^{-1}, D4= 4 plants pot^{-1} and D5= 5 plants pot^{-1}. g: Gram

Table 7. Performance of the very high tillering and dwarf rice mutants, Cocodrie and Zhe733 as influenced by five planting densities.

Genotype x Plant density	Number of productive tillers	Number of non-productive tillers	Number of total tillers	% of productive tillers	Plant height (cm)	Flag leaf length (cm)	Panicle length (cm)	Filled grains panicle^{-1}	Non-filled grains panicle^{-1}	Total grains panicle^{-1}	Grain yield (g plant^{-1})
M-13662*D1	89.11a	18.77a	107.88a	82.60	62.77f	27.21def	12.27e	18.40f	13.33e	31.73e	22.37a
M-13662*D2	40.50b	7.61bc	48.11b	84.18	64.55f	27.23def	13.38e	20.68f	12.48e	33.17e	12.32cd
M-13662*D3	30.21bcd	6.44bc	36.66bcd	82.40	63.55f	25.87def	12.93e	17.13f	14.73e	31.86e	8.09cdef
M-13662*D4	22.66cd	3.69bc	26.36cde	85.96	62.77f	24.90ef	12.34e	16.62f	12.38e	29.01e	5.60def
M-13662*D5	17.26def	3.13bc	20.40def	84.60	63.88f	23.86f	11.50e	13.13f	13.51e	26.64e	3.19ef
M-13684*D1	86.00a	20.33a	106.33a	80.88	64.77f	27.74cdef	13.58e	16.28f	18.62e	34.91e	20.44ab
M-13684*D2	42.16b	8.44b	50.61b	83.30	64.77f	26.46def	12.86e	14.93f	17.12e	32.05e	9.32cdef
M-13684*D3	34.84bc	8.40b	43.25bc	80.55	62.33f	25.32ef	12.57e	9.84f	18.00e	27.84e	5.15def
M-13684*D4	28.72bcd	4.75bc	33.47bcd	85.80	61.88f	23.46f	12.04e	8.53f	18.50e	27.03e	3.52ef
M-13684*D5	20.06de	3.86bc	23.93de	83.82	61.66f	24.12ef	11.92e	8.08f	17.46e	25.55e	2.48f
Cocodrie*D1	4.00fg	0.33c	4.33fg	92.37	111.11bcd	35.66a	23.06a	108.68a	101.14a	146.95ab	10.42cde
Cocodrie*D2	2.88fg	0.16c	3.05fg	94.42	108.88cd	29.72bcde	21.03abc	92.95ab	64.30bc	152.08a	6.73def
Cocodrie*D3	2.51g	0.25c	2.77fg	90.61	98.44e	25.88def	18.40d	70.91cd	32.09de	94.73d	4.45ef
Cocodrie*D4	2.00g	0.27c	2.27fg	88.10	98.66e	26.48def	18.90cd	84.22bc	31.58de	112.77bcd	4.15ef
Cocodrie*D5	1.68g	0.15c	1.82g	92.30	98.66e	27.82cdef	19.49bcd	86.06bc	30.68de	116.75abcd	3.60ef
Zhe733*D1	7.77efg	3.00bc	10.77efg	72.14	119.77a	33.31abc	22.60a	60.45de	78.08ab	131.77abc	14.17bc
Zhe733*D2	4.05fg	0.50c	4.55fg	89.01	117.88ab	32.99abc	23.08a	53.32de	79.02ab	127.13abcd	6.40def
Zhe733*D3	2.94fg	0.55c	3.51fg	83.76	113.77abc	34.16ab	22.18a	54.70de	62.82bc	112.68bcd	4.87ef
Zhe733*D4	2.38g	0.38c	2.77fg	85.92	108.11cd	31.42abcd	21.18abc	46.76e	61.84bc	106.08cd	3.30ef
Zhe733*D5	2.33g	0.15c	2.48fg	93.95	104.11de	35.05ab	21.97ab	44.77e	50.40cd	93.42d	3.14ef

† Within columns, means followed by a common lowercase letters are not significantly different at the 0.05 level. D1= 1 Plant hill^{-1}, D2= 2 plants hill^{-1}, D3= 3 plants hill^{-1}, D4= 4 plants hill^{-1} and D5= 5 plants hill^{-1}.
‡ g: Gram.

Across plant densities, the number of total tillers plant^{-1} of the mutants was greatly affected by different levels of nitrogen. There was a 32% and 40% increase in tiller numbers at the lower nitrogen levels as compared to the highest nitrogen level in M-13662 and M-13684, respectively. The ANOVA indicated no significant differences for productive tiller number among the three nitrogen levels, however, M-13662 and M-13684 produced the numerically highest number of productive tillers at the intermediate and lowest nitrogen levels, respectively. The number of productive tillers, non-productive tillers and total tillers were unaffected by different levels of nitrogen in controls (Table 8). Non-productive tiller production responded to nitrogen differently among cultivars (Amin et al., 2006). The same results were seen in the present study with respect to nitrogen fertilization and production of non-productive tillers.

Genotype x nitrogen level interactions were not significant for plant height, flag leaf length, panicle length, filled grains panicle^{-1}, non-filled grains panicle^{-1}, grain yield plant^{-1} and panicle exsertion length but were significant for total number of grains panicle^{-1}. The highest number of total grains panicle^{-1} was achieved at the highest nitrogen level for Zhe733 but Cocodrie was not influenced by the three levels of nitrogen. Similarly in wheat (*Triticum aestivum L.*), nitrogen increased the number of grains per spike (Khan et al., 2000; Iqtidar et al., 2006).

The production of tillers in rice is influenced by several agronomic practices, such as the planting density but these practices do not affect the formation of axillary buds (Hoshikawa, 1989; Takeda et al., 2003). Several axillary buds remain dormant at high planting density. The tiller number plant^{-1} of mutants did vary significantly under

different growing condition (nitrogen & density). In contrast, other agronomic traits, such as the height of the mutants were not affected by different levels of plant density or nitrogen levels. Considering that the recessive gene controls both plant height and tillering ability, this indicates that the expression of the gene for the tillering trait can be modified depending on the growing condition or may be there are other modifiers that contribute to tillering but its role in the control of height is fixed. Previous studies indicated that some QTLs for tillering could affect plant height (Wu. P, 1996; Yan et al., 1998). Takeda et al. (2003) concluded that rice may have additional factors other than OsTB1 that negatively regulates lateral branching or may have some positive regulator that promotes the axillary buds. Either or both (factors and regulators) may be involved in the regulatory mechanism for shoot branching related to planting density. These novel mutants, therefore, could be an important genetic resource to study the molecular pathway related to tillering in rice and other grasses.

Table 8. Performance of the very high tillering dwarf rice mutants, Cocodrie and Zhe733 as influenced by three levels of nitrogen.

Genotype x Nitrogen level	Number of productive tillers	Number of non-productive tillers	Number of total tillers	% of productive tillers	Plant height (cm)	Flag leaf length (cm)	Panicle length (cm)	Filled grains panicle^{-1}	Non-filled grains panicle^{-1}	Total grains panicle^{-1}	Grain yield (g plant^{-1})
M-13662*N1	38.64a	8.01bc	46.66abc	82.81	64.53c	26.27cd	12.56d	15.94c	13.01d	28.96c	9.04ab
M-13662*N2	46.14a	9.04abc	55.18ab	83.61	62.33c	24.66d	12.30d	18.06c	13.05d	31.12c	12.84a
M-13662*N3	35.06a	6.73bcd	41.80c	83.87	63.66c	26.51cd	12.59d	17.57c	13.80d	31.38c	9.07a
M-13684*N1	45.61a	13.93a	59.54a	76.6	62.13c	23.36d	12.60d	11.44c	17.44d	28.88c	8.09ab
M-13684*N2	42.57a	9.88ab	52.45abc	81.16	62.86c	26.38cd	12.10d	11.29c	17.63d	28.92c	9.08ab
M-13684*N3	38.90a	3.65cde	42.55bc	91.42	64.26c	26.52cd	13.08d	11.88c	18.74d	30.62c	7.36b
Cocodrie*N1	2.59c	0.10e	2.70d	95.92	102.53b	29.09bc	19.95c	83.23a	45.93c	112.21ab	5.56b
Cocodrie*N2	2.66c	0.34e	3.00d	88.66	104.93b	29.39bc	20.21bc	87.40a	55.68bc	134.38a	5.74b
Cocodrie*N3	2.59c	0.26e	2.85d	90.87	102.00b	28.85bc	20.38bc	95.08a	54.27bc	127.38a	6.30b
Zhe733*N1	3.38c	0.77e	4.16d	81.25	112.86a	34.04a	21.38bc	44.38b	66.56ab	101.17b	4.69b
Zhe733*N2	3.96c	1.32de	5.29d	74.85	110.80a	32.08ab	21.98ab	54.34b	59.06abc	110.53ab	6.83b
Zhe733*N3	4.35c	0.65e	5.01d	86.82	114.53a	34.03a	23.25a	57.28b	73.68a	130.96a	7.61ab

† Within columns, means followed by a common lowercase letters are not significantly different at the 0.05 level. N1= 179 kg ha^{-1}, N2= 202 kg ha^{-1} and N3= 224 kg ha^{-1}.
‡ g: Gram.

CHAPTER V

SUMMARY AND CONCLUSIONS

Tillering and height in rice are important agronomic traits, which determine the yield potential of a particular cultivar. Tillering ability is affected by environmental conditions such as light, temperature, plant density, and nutrients and genotypes. Sixteen very high tillering and dwarf rice mutant lines along with Cocodrie, and Zhe733 (controls) were planted in a field at the Texas AgriLife Research and Extension Center, Beaumont, Texas. Phenotypic characterization was done for all the mutant lines. Two mutant lines (M-13662 & M-13684) were further selected to determine the effect of three different levels of nitrogen, and five different planting densities on different agronomic traits, and the effect of GA application on second leaf sheath elongation was assessed. Crosses including reciprocals were made with Cocodrie to determine the inheritance pattern of the gene controlling very high tillering and dwarf traits in the mutant. The novel rice mutants were characterized by their dwarf stature (50-55 cm) and their bushy phenotype due to the production of fine culm tillers (89-121 tillers plant^{-1}). Reduced elongation of the top four internodes and the compression of the basal 2-3 internodes accounted for their dwarf stature. The rice mutants were similar to the *d* mutants in terms of plant height (40-65 cm). The mutant lines produced tillers faster than semi-dwarf conventional cultivars. The first tiller of the mutant emerged at the 4th leaf stage whereas, no tiller was observed in Cocodrie at the same leaf stage. The first active lateral bud under the first leaf sheath at the 3rd leaf stage of mutant was observed in this study. Active buds were observed in each leaf axil until the 7th leaf stage in both mutants

and Cocodrie but only 2-3 lateral buds in the main culm of mutants were able to produce primary tillers. The rate of tiller production was doubled every week until seven weeks after emergence of the first tiller but the rate of tiller production was slow between the 13^{th} to 16^{th} weeks after emergence of the first tiller. However, after this period, the mutants started producing tillers at the rate similar to that observed during the first seven weeks after emergence, which resulted in a final tiller number that was nearly 6-8 times and 4-5 times of the tillers in Cocodrie and Zhe733, respectively. In Cocodrie and Zhe733, the production of new tillers became static after the panicle initiation stage but the mutants kept producing new tillers even at the late reproductive stage. The mutants were late in heading and maturity as compared to both controls (Cocodrie and Zhe733) and were not ready for harvest until 26 days later. Panicles were very short (12-13 cm) with few grains $panicle^{-1}$ (25-30) as compared to controls. Since the mutant had a small number of grains $panicle^{-1}$, shorter plant stature and duration of grain filling, and the leaves remain green at harvest, much of the mobile carbohydrates, protein and mineral nutrients from different sources were likely not transported and remained in the stem and leaves. These resources were likely used to produce the large number of tillers even at the late reproductive stage. Generally, dwarf and high tillering traits are associated in all high tillering dwarf mutants of rice, and the same associated traits were observed in our rice mutants. Field observation and destructive sampling suggested that the production of high tiller numbers was the result of the release of axillary buds from a dormant stage rather than initiation of additional axillary buds. Each tiller of the mutant had the capacity to serve as a mother tiller for further tiller production whereas only the main

culm, primary and secondary tiller of wild type plants produced new tillers. Reduced apical dominance might be one of the factors contributing towards the dwarf stature of the very high tillering dwarf rice mutants. Other agronomic traits like flag leaf length, panicle length, filled grains panicle^{-1}, total grains panicle^{-1}, seed size and 1000 grain weight were found to be reduced compared to the controls. The mutants were classified into a typical long grain category based on seed length-width ratio (> 3.00:1). The mutant had similarities to the *d* mutants (*d3, d10, d14, d1 and d27*) from the early stage of development in terms of high number of fine tillers, short stature and small grain size. However, the average shortening of the top four internodes observed in the very high tillering dwarf rice mutants indicated its similarity to the *htd-1* mutant. The internode elongation pattern of the mutant followed the dn-type dwarf pattern based on Takeda's classification but a few plants had different dwarfing patterns, which were not included in the classification.

Across different densities and N levels, both rice mutants (M-13662 & M-13684) were similar to each other but significantly different from conventional rice cultivars with the *sd-1* semi-dwarf gene (Cocodrie & Zhe733) for all studied agronomic traits. Five different planting densities as well as three different nitrogen levels affected the tillering capacity of mutants. More tillers (productive, non-productive and total tillers) were observed at 179 kg ha^{-1}. There were no significant differences observed between 179 and 202 kg ha^{-1} for total tiller number. Different planting densities as well as nitrogen levels did not affect height of the mutants (M-13662 & M-13684). Variation in flag leaf length, panicle length, filled grains panicle^{-1}, non-filled grains panicle^{-1} and

total grains panicle^{-1} were not significant for different planting densities and nitrogen level in both mutants but the grain yield plant^{-1} was highly significant for both mutants and Zhe733 cultivar.

Both mutants were considered GA responsive based on a GA bioassay. The segregation ratio of F_2 populations showed that the very high tillering and dwarf traits were likely controlled by a single recessive gene.

Different kinds of rice mutants have vital roles in forming the basis of genetic analysis and functional genomics studies and hundreds of increased tillering dwarf rice mutants are included in the mutant collection. However, most of them are uncharacterized (Ishikawa et al., 2005). Studies have indicated that rice tillering is a complex process and is controlled by genes and QTL (Miyamoto et al., 2004; Ishikawa et al., 2005; Zou et al., 2005). The identification, characterization and genetic analysis of new novel mutants are initial steps to elucidate the molecular mechanism and gene relationships related to the expression of the mutant traits. The characterization of the very high tillering dwarf mutant will be important in understanding the mechanisms that control tiller formation in rice or shoot branching in other crop plants. Such characterization may also help unravel the mechanisms involved in the determination of plant height in rice and its relationship to tiller growth and development.

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